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NUCLEAR DIVISIONS IN THE POLLEN MOTHER CELLS OF TRITICUM, AEGILOPS, AND SECALE AND THEIR HYBRIDS[1]

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INTRODUCTION

The phylogenesis of wheat has been a productive field of speculation and investigation, particularly since the early part of the nineteenth century. The various theories proposed to explain the origin of wheats have been based principally on the results of taxonomic studies. According to one of the views all of the species of Triticum have originated from the wild prototype *Triticum aegilopoides*. Another view is that the wheats are polyphyletic and that *T. aegilopoides* and *T. dicoccoides* are the wild prototypes of the einkorn and emmer groups, respectively, while the prototype of the *vulgare* group is still unknown. A more recent view derives the species of the latter group (*vulgare*) by hybridization of members of the emmer wheat groups with *Aegilops cylindrica* and *A. ovata*. The ease with which it is possible to cross the three genera Triticum, Secale, and Aegilops suggests a relationship between them which upon further investigation may definitely establish the origin of the wheats. The development of cytology has afforded the opportunity for an analysis of the problem from a new angle. A recent review of the literature on this subject by Bleier (*6*) [2] indicates the extent to which cytology has been recognized in analyzing the genetical material accumulated by investigators interested in this problem.

The results of cytological studies of the genera Triticum, Secale, Aegilops, and some of their hybrids are presented in this paper.

MATERIAL AND METHODS

The species of Aegilops, Secale, and Triticum used in making the hybrids herein reported were obtained from various sources. Two varieties of *A. crassa*, namely, *rubiginosa* and *rufescens*, were obtained from C. O. Johnston, engaged in cooperative leaf-rust investigations of the United States Department of Agriculture and the Kansas Agricultural Experiment Station at Manhattan, Kans., who received them from the Russian Bureau of Applied Botany. The remainder of the species of Aegilops were obtained through the Office of Foreign Plant Introduction, United States Department of Agriculture. With

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[2] Reference is made by number (italic) to "Literature cited," p. 717.

the exception of *A. crassa*, the species of Aegilops studied, or used in making hybrids, agree with the descriptions of those given by Ascherson and Graebner (*2*).

The material of *Secale montanum* used in these studies was obtained from E. B. Mains, engaged in cooperative leaf-rust investigations of the United States Department of Agriculture and Purdue University Agricultural Experiment Station, at La Fayette, Ind. The forms of Triticum and two varieties of *S. cereale* used are from the collection of the Office of Cereal Crops and Diseases.

All the crosses were made by the junior writer. The F_1 plants and parents were grown in the greenhouses at Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C. All of the F_1 plants were grown side by side in the same greenhouse under as nearly identical conditions as possible, thus reducing to a minimum the chance of one hybrid being more favorably situated than another. This procedure seemed a desirable precaution against a possible unfair comparison, particularly in those individuals whose loosely paired chromosomes may be subject to change in behavior under different environments.

Cytological studies were made on the following F_1 hybrids:

Female		*Male*
T. dicoccoides var. *aaronsohni*	×	*T. monococcum* var. *flavescens.*
T. turgidum var. C. I. 7821	×	*T. monococcum* var. *flavescens.*
T. polonicum var. C. I. 7070	×	*T. monococcum* var. *flavescens.*
T. compactum var. Dale Gloria	×	*T. monococcum* var. *flavescens.*
T. vulgare var. C. I. 7843	×	*T. monococcum* var. *flavescens.*
T. vulgare var. C. I. 7959	×	*T. monococcum* var. *flavescens.*
T. spelta var. Alstroum	×	*T. monococcum* var. *flavescens.*
T. dicoccoides var. *aaronsohni*	×	*S. montanum.*
T. vulgare var. Fulhio	×	*S. montanum.*
T. vulgare var. Dawson × Kanred	×	*S. montanum.*
T. spelta var. Alstroum	×	*S. montanum.*
T. vulgare var. Gipsy	×	*S. cereale.*
S. cereale	×	*S. montanum.*
A. cylindrica var. *rubiginosa*	×	*T. dicoccoides* var. *aaronsohni.*
A. cylindrica var. *rubiginosa*	×	*T. polonicum* var. C. I. 7842.
A. crassa var. *rubiginosa*	×	*T. dicoccoides* var. *aaronsohni.*
A. crassa var. *rufescens*	×	*T. dicoccum* var. Black Winter.
A. crassa var. *rufescens*	×	*T. dicoccum* var. Khapli.
A. crassa var. *rufescens*	×	*T. durum* var. C. I. 7789.
A. crassa var. *rufescens*	×	*T. turgidum* var. Alaska.
A. crassa var. *rufescens*	×	*T. polonicum* var. C. I. 7842.
A. crassa var. *rufescens*	×	*T. compactum* var. Dale Gloria.
A. crassa var. *rufescens*	×	*T. vulgare* var. Purple Straw.
A. crassa var. *rubiginosa*	×	*T. spelta* var. Alstroum.
A. triuncialis	×	*A. ovata.*

The F_3 plants of the cross *A. ovata* × *T. dicoccum* var. Black Winter studied cytologically were grown under conditions similar to those of the F_1 plants referred to above. Several heads of each of the F_1, F_2, and F_3 plants of this cross were carefully inclosed in glassine bags or isolated before pollination occurred, thus preventing any opportunity for contamination by cross-pollination.

After making a preliminary examination of the pollen mother cells to determine the proper stage of development, the junior writer collected the young heads of the various species and hybrids and put them immediately into vials of acetic absolute (1–3) killing fluid. At the end of 20 to 30 minutes the killing fluid was poured off and the heads were covered with absolute alcohol. This alcohol was later

changed several times, until no odor of acetic acid was detected. The vials were then corked tightly and put away for future study.

The collections were made in the spring of 1927 and studied in the laboratory during the following winter.

The anthers were dissected out and put on a slide with a few drops of aceto-carmine staining fluid. The anthers were then cut open and pressed lightly, forcing the pollen mother cells out into the liquid. The fragments of the anther walls were next removed with the edge of a cover glass, leaving the cells floating in the liquid. The cover glass was placed carefully on the slide to prevent the cells from floating out beyond the edge of the cover glass. The preparation was then allowed to remain in this condition until sufficient liquid had evaporated to permit the cover glass to fit snugly on the slide. When suitable material was obtained, the edges were sealed with paraffin to prevent further evaporation of the liquid. A slide prepared and sealed in this manner often kept satisfactorily for a week or more.

Material was examined with a Leitz $\frac{1}{16}$-oil-immersion objective and × 8, × 10, and × 15 oculars. Camera-lucida drawings were made of a large number of stages, a continuous series of pictures of the various meiotic phases being obtained when possible. In addition to drawings a large number of photomicrographs were taken with a Leitz microcamera. The magnifications are shown in the legends.

CHROMOSOMES IN SECALE CEREALE

The chromosome number for *Secale cereale* was definitely established as 7 by Sakamura (*33*). Prior to this time Nemec (*25*) reported 12 as the diploid number, and Spillman (*43*) stated that the haploid number is 6, "I believe." Nakao (*24*), on the other hand, found 8 as the reduced number.

The work of recent investigators, as shown in Table 1, has substantiated Sakamura's determination of 7 as the chromosome number for this species. Stolze (*44*) found 7 to be the reduced chromosome number for *Secale montanum* also. Several of these investigators, however, found plants of *S. cereale* with 7 + 1 as the haploid number. This number is likely to arise from the diploid species when nondisjunction causes an unequal distribution of the chromosomes in the reduction phases. Under such circumstances the pollen and egg cells will have extra chromosomes.

TABLE 1.—*Chromosome numbers of Secale cereale and S. montanum as determined by the investigators named*

[Diploid chromosome numbers in figure columns are shown in italics; all other numbers are haploid chromosomes]

Investigator	S. cereale	S. montanum	Investigator	S. cereale	S. montanum
Nemec, 1910 (*25*)	*12*		Stolze, 1925 (*44*)	*14*, 7	{ 6 { *14*, 7
Spillman, 1911 (*43*)	6				
Nakao, 1911 (*24*)	8		Belling, 1925 (*5*)	{ 7	
Sakamura, 1918 (*33*)	*14*, 7			{ 8	
Ferrand, 1923 (*9*)	7		Aase and Powers, 1926 (*1*)	7	
Kihara, 1924 (*19*)	{ *14*, 7		Thompson, 1926 (*46*)	7	
	{ *16*, 8		Present writers	7	
Nikolaewa, 1924 (*28*)	7				
Gotoh, 1924 (*13*)	{ 7				
	{ 8				

In the present study it was found that *Secale cereale*, var. Abruzzes, has 7 as its reduced chromosome number. Figure 1, A, B, and C,

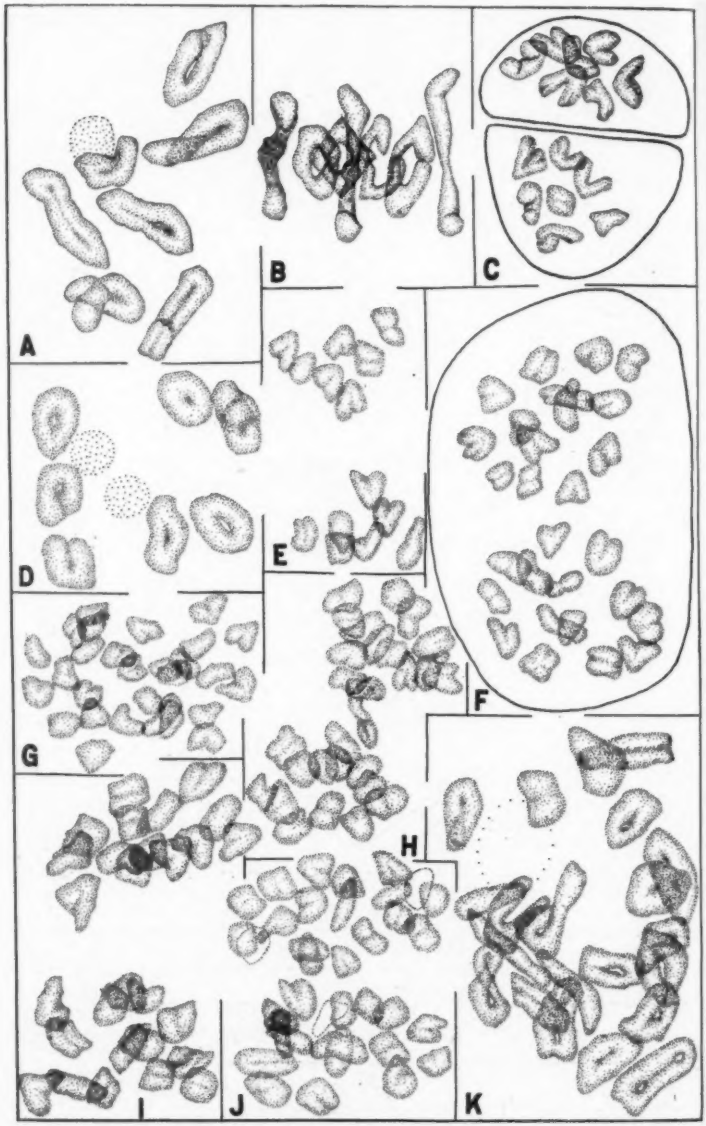


FIGURE 1.—Chromosomes in Secale and Triticum species. × 2,000: A, Diakinesis in *S. cereale* L.; B, heterotypic metaphase in *S. cereale* L., showing the character, position, and shape of the chromosomes at this phase; C, homotypic metaphase in *S. cereale* L.; D, diakinesis in *T. monococcum* L.; E, late heterotypic anaphase in *T. monococcum* L.; F, late heterotypic anaphase in *T. dicoccoides* Kcke.; G, heterotypic anaphase in a regular form of *T. polonicum* L.; H, heterotypic anaphase in *T. dicoccum* Schrk.; I, heterotypic anaphase in *T. turgidum* L.; J, heterotypic anaphase of an apparently fixed segregate of *T. vulgare* × *T. polonicum*, showing 33(–36?) chromosomes; K, diakinesis in *T. compactum* Host.

shows three reduction phases. The chromosomes are all paired at diakinesis and are distributed regularly in practically every figure studied.

Considerable attention was given to the shape of the individual chromosomes as they appeared on the heterotypic metaphase plate. Figure 2 is a diagrammatic representation of the 7 chromosomes at this phase. Three of the chromosomes, A, B, and C, have only slightly subterminal points of fiber attachment. At the late metaphase the halves of these three chromosomes are stretched apart and seem much longer than chromosomes D, E, F, and G. The latter have almost central points of fiber attachment, vary in shape from the open C type to the closed double V, and appear much shorter than chromosomes A, B, and C. Figure 1, B, shows the 7 chromosomes in a characteristic position. The two halves of the 3 chromosomes with subterminal fiber attachment appear as rods or J's and are easily distinguished from the other 4 chromosomes. In the early anaphase

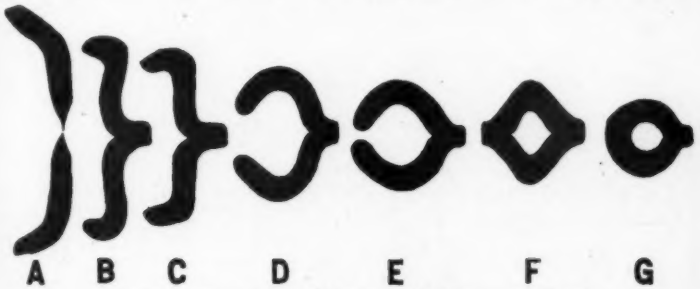


FIGURE 2.—Characteristic shapes of individual chromosomes in rye

stage all halves of the chromosomes D, E, F, and G are V-shaped and show only slight differences in the points of fiber attachment. Gotoh (*13*) presents similar pictures for Secale chromosomes. The differences between the shapes of the chromosomes illustrated by Gotoh and those pictured by the writers probably are due to the variable positions assumed by the chromosomes while under tension during division.

CHROMOSOMES IN F_1 HYBRIDS OF SECALE CEREALE× S. MONTANUM

The haploid chromosome number of *Secale cereale* is 7, with few exceptions. Stolze (*44*) has found the same number for *S. montanum*. The F_1 hybrid which provided cytological material for the writers' study has 7 chromosomes as the reduced number, as was anticipated.

The generally observed behavior of the chromosomes in the meiotic phases of the developing pollen mother cell of the F_1 hybrid studied by the writers was regular (fig. 3, A), giving tetrads with 7 chromosomes in each cell. Occasionally abnormal chromosome behavior was observed in diakinesis and in the heterotypic anaphase. In diakinesis a few unpaired chromosomes may be observed distributed among the bivalents. In the heterotypic anaphases six bivalent and two univalent chromosomes were sometimes seen. Under such conditions the following variable distribution of the univalent chromosomes may occur: (1) Both univalent chromosomes pass undivided to one pole

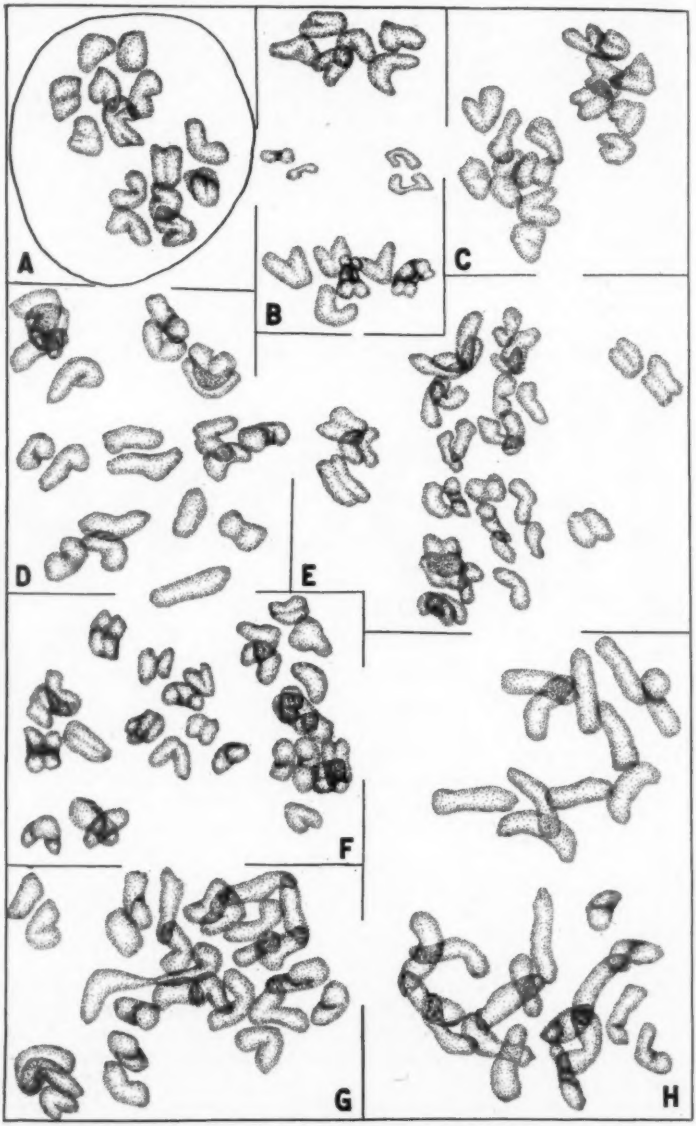


FIGURE 3.—Chromosomes in Secale × Secale and Secale × Triticum hybrids. × 2,000; A, Late heterotypic anaphase in *S. cereale* × *S. montanum;* B, late heterotypic anaphase in *S. cereale* × *S. montanum*, showing 6 chromosomes at each pole and 2 split univalents on the spindle; C, heterotypic anaphase in *S. cereale* × *S. montanum*, showing 6 chromosomes going to one pole and 8 to the other; D, heterotypic prophase in *T. dicoccoides* × *S. montanum*, showing 21 univalent chromosomes; E, heterotypic metaphase in *T. dicoccoides* × *S. montanum*, showing a few chromosomes at each pole and splitting univalents on the equatorial plate; F, heterotypic anaphase in *T. vulgare* × *S. montanum*, showing a few chromosomes at the equatorial plate region; G, heterotypic metaphase in *T. vulgare* × *S. montanum*, showing a bivalent chromosome; H, heterotypic prophase in *T. vulgare* × *S. cereale*, showing 28 unpaired chromosomes

(fig. 3, C); (2) one chromosome passes to each pole; (3) the two univalents split longitudinally and tardily join the earlier divided chromosomes, giving to each nucleus two types of chromosomes (fig. 3, B); (4) one univalent splits, one half passing to each pole, while the other univalent goes undivided to either pole.

The homotypic division was found to be regular if no halves of univalents were in the chromosome complement. In the homotypic anaphase the halves when present usually lag behind the divided chromosomes and pass at random without further division to the daughter nuclei.

The pollen nucleus of this hybrid usually has seven chromosomes, but the number may be either above or below this basic number, due to such irregularities as those described above.

CHROMOSOMES IN TRITICUM SPECIES

A general study of chromosomes in wheat species was made in conjunction with that of the Triticum hybrids.

The chromosome number for most of the Triticum species has been definitely established. The determinations of the various investigators are listed in Table 2.

The chromosomes of the various species used by the writers are shown in Figure 1, D–I and K, and Figure 4, D and E. A heterotypic anaphase of *Triticum polonicum*, with 14 chromosomes nearing each pole, is shown in Figure 1, G. This number confirms the studies of other investigators who place *T. polonicum* among the tetraploid species. Another form of wheat (F. S. P. I. No. 56233) that had the long glumes of Polish and the lax head and tough glumes of spelt was also studied. This wheat was received from Portugal with a pedigree showing that it had originated as a cross between *vulgare* and *polonicum* wheats. Judging from morphological characters, it is apparently a fixed segregate from this cross. Figure 1, J, shows a heterotypic anaphase of this odd form with 17 chromosomes at one pole and 16 at the other. In addition to these chromosomes two extra questionable chromosomes may be observed at one pole and one chromosome at the other, giving the reduced number of at least $\frac{33}{2}$ and possibly as high as 18. A study of the homotypic anaphases shows a few chromosomes irregularly distributed to the poles or extruded into the cytoplasm. As a result of the latter phenomenon many cells in the pollen tetrads have one or more minor nuclei in addition to the major nucleus. It is interesting to find a form apparently fixed with a chromosome number intermediate between that of the two parents. Several crosses were made involving this form, but no hybrids were studied.

Photomicrographs of six meiotic phases of the developing pollen mother cell in Triticum species are shown in Plate 1, A–F. They are arranged in sequence and show the regular distribution of chromosomes to the daughter cells. Plate 1, A, is a heterotypic prophase of *T. monococcum* showing seven bivalent chromosomes and the nucleolus. Plate 1, B, shows a typical heterotypic metaphase of *T. vulgare;* C shows an anaphase of *T. vulgare* with a few chromosomes dividing; D shows a heterotypic telophase of *T. vulgare* with two equal-sized chromatin masses at each pole; E shows homotypic telophases of *T. compactum* in which the chromosomes have divided and moved to the poles in a regular manner; F is a typical tetrad of *T. spelta.*

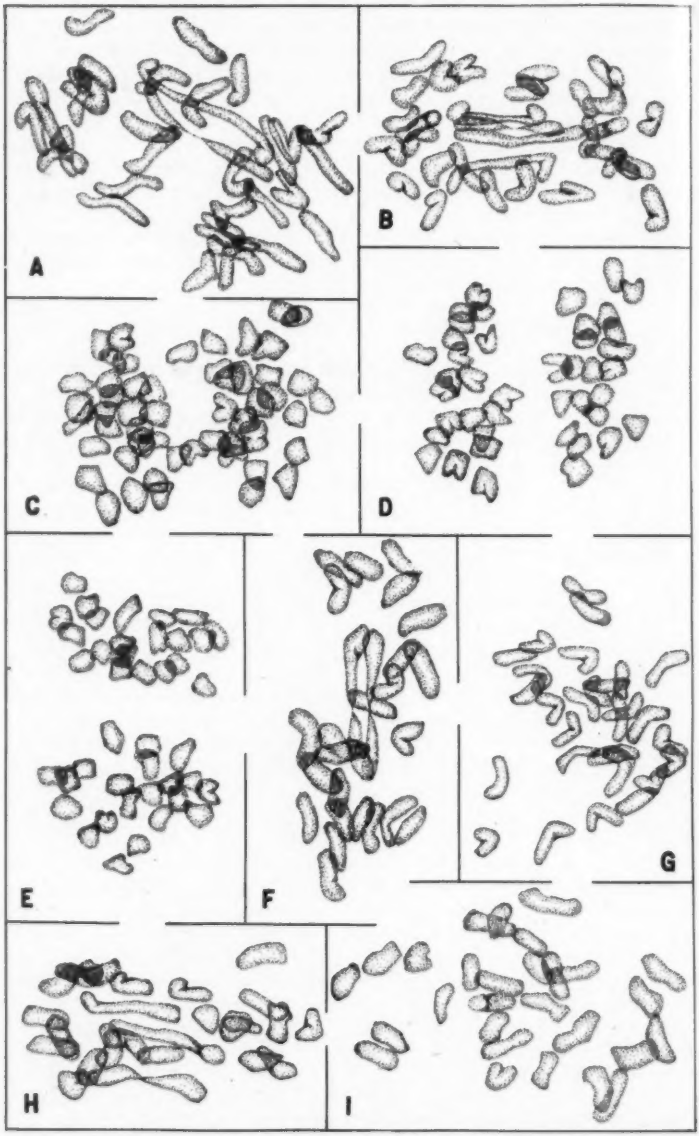


FIGURE 4.—Chromosomes in Triticum species and Aegilops × Triticum hybrids. × 2,000: A, Heterotypic metaphase in *A. crassa* × *T. dicoccum;* B, heterotypic metaphase in *A. crassa* × *T. turgidum;* C, heterotypic anaphase in F_1 plant of *A. ovata* × *T. dicoccum;* D, heterotypic anaphase in *T. spelta* L.; E, heterotypic anaphase in *T. vulgare;* Vill.; F, heterotypic metaphase in *A. cylindrica* × *T. turgidum;* G, heterotypic prophase in *A. cylindrica* × *T. turgidum;* H, heterotypic metaphase in *A. cylindrica* × *T. polonicum;* I, heterotypic prophase in *A. cylindrica* × *T. polonicum*

TABLE 2.—*Chromosome numbers of some Triticum species as determined by the investigators named*

[Diploid chromosome numbers in figure columns are shown in italics; all other numbers are haploid chromosomes]

Investigator	T. aegilopoides	T. monococcum	T. thaoudar	T. dicoccoides	T. dicoccum	T. durum	T. turgidum	T. persicum	T. pyramidale	T. polonicum	T. vulgare	T. compactum	T. sphaerococcum	T. spelta
Overton, 1893 (*19*)											*16*, 8			
Goloiński, 1893 (*12*)											*16*, 8			
Koernicke, 1896 (*21*)												*16*, 8		
Spillman, 1911 (*48*)											[a] *40+*			
Nakao, 1911 (*24*)											*16*, 8			
Bally, 1912 (*5*), 1919 (*4*)				8							8 or 9			
Sakamura, 1918 (*33*)		*14*			*28*	*28*	*28*			*28*	*42*, 21	*42*		*42*
Sax, 1918 (*35*), 1921 (*36*), 1922 (*37*)		7			14	*28*, 14	14			14	21	21		21
Nikolaewa, 1922 (*26*), 1922–23 (*27*)		*14*			*28*	*28*	*28*	*28*		*28*	[b] *42+*	[c] *42+*	[b] *42+*	[c] *44+*
Zhukovsky, 1923 (*51*)								*28*						
Watkins, 1924–25 (*48*)		*14*			*28*	*28*	*28*			*28*	*42*	*42*		*42*
Winge, 1924 (*49*)											*42*, 21			
Kihara, 1924 (*18*)	*14*, 7	*14*, 7		*28*, 14	*28*, 14	*28*, 14					*42*, 21	*42*, 21		*42*, 21
De Mol, 1924 (*33*)		7		[d] 7	14	14	14			14	21	21		21
Stolze, 1925 (*44*)	*14*			14	14						*42*			21
Kagawa, 1927 (*14*, *15*), 1928 (*16*)		*14*		*28*	*28*	*28*	*28*			*28*	*42*	*42*, 21		
Tschermak and Bleier, 1926 (*47*)				14		14								
Thompson, 1926 (*45*, *46*)		7					14				21			
Flaksberger, 1926 (*10*)			*14*, 7	*28*, 14										
Percival, 1926 (*32*)	7	7		14	14	14	14		14	14	21	21		21
Aase and Powers, 1926 (1)		7		14	14	14	14				21	21		21
Gaines and Aase, 1926 (11)												21		
Present writers		7		14	14		14			14	21	21		21

[a] 40 or more.
[b] 42 to 44.
[c] 44 to 50.
[d] According to Flaksberger (*10*), De Mol's *T. dicoccoides* should be *T. aegilopoides*.

CHROMOSOMES IN F_1 HYBRIDS OF TRITICUM

Numerous investigators have studied the chromosomes in interspecific wheat hybrids. Table 3 lists 26 crosses between different species of wheat in which the chromosome number and behavior have been described.

The present study traces the chromosome behavior of certain Triticum × Triticum hybrids through many of the important meiotic phases of developing pollen mother cells. These include crosses between *T. monococcum* and species of the emmer group resulting in triploid hybrids, and crosses between *T. monococcum* and species of the *vulgare* group resulting in tetraploid hybrids. *T. monococcum* was used as the pollen parent in crosses with both groups of Triticum.

TABLE 3.—*Summary of cytological work on F_1 interspecific hybrids of Triticum*

Hybrid combination		Chromosome number of parents		Chromosome pairing	Authority	Year
Female	Male	Female	Male			
T. aegilopoides	T. dicoccum	7	14	4–7	Kihara (*19*)	1924
Do	do	7	14		Kihara and Nishiyama (*20*)	1928
T. monococcum	T. Turgidum	7	14	(?)7	Sax (*37*)	1922
Do	do	7	14	3–7	Thompson (*45*)	1926
T. dicoccoides	T. monococcum	14	7	0–6	Present writers	
T. dicoccum	do	14	7	4–7	Kihara (*19*)	1924
Do	do	14	7		Kihara and Nishiyama (*20*)	1928
Do	T. vulgare	14	21	14	Sax (*38*)	1923
T. durum	do	14	21	14	Kihara (*17, 18, 19*)	1919, 1921, 1924
Do	do	14	21	14	Sapehin (*34*)	1928
Do	do	14	21	14	Sax (*37*)	1922
Do	do	14	21		Kihara and Nishiyama (*20*)	1928
T. turgidum	T. monococcum	14	7	0–7	Present writers	
Do	T. compactum	14	21	14	Kihara (*17, 19*)	1919, 1924
Do	T. vulgare	14	21	14	Watkins (*48*)	1924–25
T. polonicum	T. monococcum	14	7	0–5	Present writers	
Do	T. compactum	14	21	14	Kihara (*19*)	1924
Do	T. spelta	14	21	14	Kihara (*17, 19*)	1919, 1924
T. vulgare	T. monococcum	21	7	0–7	Present writers	
Do	T. durum	21	14	14	Sax (*37*)	1922
Do	T. turgidum	21	14	14	do	1922
T. compactum	T. monococcum	21	7	0–7	Present writers	
Do	T. durum	21	14	14	Sax (*37*)	1922
T. spelta	T. aegilopoides	21	7		Kihara and Nishiyama (*20*)	1928
Do	T. monococcum	21	7	0–5	Melburn and Thompson (*22*)	1927
Do	do	21	7	0–5	Present writers	

TRIPLOID HYBRIDS

The abundant cytological material available from the cross *Triticum dicoccoides* × *T. monococcum* made it possible to obtain a continuous picture of the chromosome behavior through both reduction divisions. Characteristic prophases showed both univalent and bivalent chromosomes. The two types of chromosomes are distinguished most easily when the latter are on the equatorial plate, for at this time the univalents are scattered through the nuclear region. A count of 9 cells taken at random gave 1 with 6 bivalents and 9 univalents, 4 with 5 bivalents and 11 univalents, 3 with 4 bivalents and 13 univalents, and 1 with 3 bivalents and 15 univalents. These few counts represent

the combinations most commonly observed, though occasionally less pairing than this may be seen. In no cases were cells observed with more than six paired chromosomes. The marked variability in the number of paired and unpaired chromosomes indicates that no definite number of paired chromosomes is characteristic of this hybrid.

Figure 5, A, shows a heterotypic metaphase, if this phase can be said to exist, with 5 bivalent chromosomes on the plate and 11 univalent chromosomes scattered through the spindle region. In B, which shows a later phase, the bivalents are moving toward the poles and have joined a few of the univalents that failed to move to the equatorial plate. Four univalents are shown just prior to splitting longitudinally. A later stage is shown in D, in which the late-dividing univalents are moving in two groups to the poles. C shows a late anaphase with four groups of chromosomes in their characteristic positions. They are frequently seen arranged in this manner prior to the formation of the two daughter nuclei.

Pollen mother cells undergoing the second reduction division usually appear abnormal, due to the presence of halves of univalent chromosomes in each chromosome group. The halves of bivalents and the undivided univalent chromosomes divide and move to the poles in a regular manner. In the homotypic anaphase the halves of univalent chromosomes that split in the previous division are frequently found lagging on the spindle, as shown in Figure 5, E, and go at random without further division to the poles or lag behind and fail to be included in the daughter nuclei, in which case they form micronuclei. The pollen tetrad shown in Figure 5, F, shows each cell with a major nucleus and from one to several minor nuclei. The number of minor nuclei depends to a large degree on the number of chromosomes that fail to be included in the major nucleus. In 379 tetrads only 2 were found in which all 4 pollen grains were without supernumerary nuclei. In a study of 102 pollen grains 37 had one nucleus only, 49 had a major and a minor nucleus, 13 had a major and 2 minor nuclei, 2 had a major and 3 minor nuclei, and 1 had a major and 4 minor nuclei.

The chromosome behavior in the meiotic phases of the pollen mother-cell development in *Triticum turgidum* × *T. monococcum* was similar to that described for the previous cross. There seemed to be a somewhat greater tendency for chromosomes to pair in this hybrid. Figure 5, H, shows a metaphase with seven bivalents in the plate region, but other cases were observed showing only unpaired chromosomes. Figure 5, I, shows a later phase, with the divided bivalents at the poles, and nine univalents on the equatorial plate in the process of splitting longitudinally.

The second division shows the same random assortment of the split univalent chromosomes as was described for the previous cross. Many of these chromosomes remain in the cytoplasm and form micronuclei.

The third cross, *Triticum polonicum* × *T. monococcum*, shows in the early heterotypic phases four or five paired chromosomes as the most common combination. (Fig. 5, G.) The behavior of the univalents is similar to that of the above-described crosses. It is unusual to find at the end of this division any indications that chromosomes have been left in the cytoplasm.

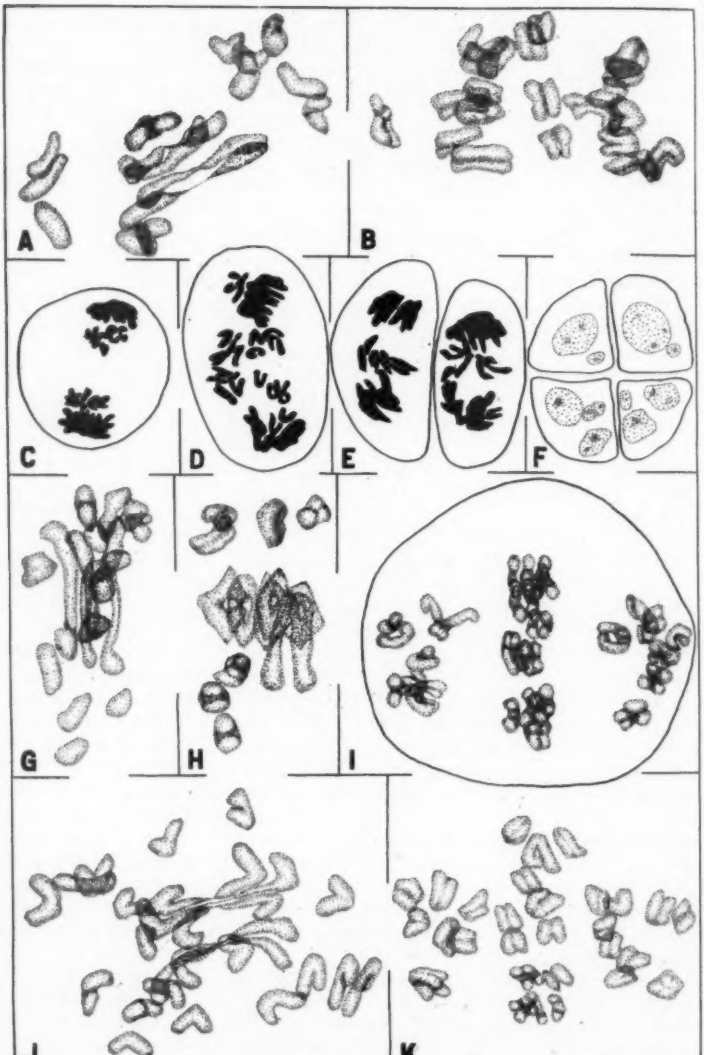


FIGURE 5.—Chromosomes in Triticum × Triticum hybrids: A, Heterotypic metaphase in *T. dicoccoides* × *T. monococcum;* B, heterotypic anaphase in *T. dicoccoides* × *T. monococcum;* C, late heterotypic anaphase in *T. dicoccoides* × *T. monococcum;* D, heterotypic anaphase in *T. dicoccoides* × *T. monococcum;* E, homotypic anaphase in *T. dicoccoides* × *T. monococcum;* F, tetrad in *T. dicoccoides* × *T. monococcum;* G, heterotypic metaphase in *T. polonicum* × *T. monococcum*, showing four bivalents and two univalents in the plate region; H, heterotypic metaphase in *T. turgidum* × *T. monococcum;* I, late heterotypic anaphase in *T. turgidum* × *T. monococcum;* J, heterotypic metaphase in *T. compactum* × *T. monococcum;* K, heterotypic anaphase in *T. compactum* × *T. monococcum*. A, B, G–K, × 2,000; C–F, × 875

A characteristic phenomenon of triploid hybrids is the formation of tetrads, each cell of which may contain, besides a major nucleus, one or more minor nuclei. The presence of minor nuclei in the cells of a tetrad suggests that in the heterotypic division some univalent chromosomes split longitudinally and are included in the two daughter nuclei, while in the homotypic division several split univalents fail to be included in the major nuclei and form micronuclei.

Sax *(37)* was the first to describe the chromosome behavior in developing pollen mother cells of the triploid wheat hybrid *Triticum monococcum* × *T. turgidum*. Thompson (*45*) also studied the cross but failed to confirm Sax's report that seven bivalents usually are present in the early heterotypic phase and that the univalents go undivided to the poles in the first division. The results of this study support Thompson's work and show the number of paired chromosomes to be variable, with some univalents splitting longitudinally in the heterotypic division. Kihara (*19*) described the behavior of chromosomes during the meiotic divisions in the hybrids of two other triploid crosses, *T. aegilopoides* × *T. dicoccum* and *T. dicoccum* × *T. monococcum*. His results are similar to those of Thompson and those of the writers herein described except that he found some chromosomes lost in the cytoplasm in the first division. The more recent chromosome study by Kihara and Nishiyama (*20*) of triploid, tetraploid, and pentaploid wheat hybrids shows the presence of trivalent chromosomes, a combination not observed by the writers in any of the material studied.

TETRAPLOID HYBRIDS

The hybrid *Triticum compactum* × *T. monococcum* gave abundant material for a study of the important phases in the reduction division of the pollen mother cells. A variable number of univalent and bivalent chromosomes was observed. Figure 5, J, shows an early heterotypic phase with 5 bivalent and 18 univalent chromosomes. Other cells, however, show as high as 7 paired chromosomes, while a few indicate no chromosome pairing. Figure 5, K, shows a later phase with 12 univalent chromosomes on the equatorial plate and groups of chromosomes near each pole composed of the divided bivalents and a few univalents that failed to move into the plate region. Later stages show that the univalent chromosomes which go to the equatorial plate split and the halves move to the poles to join the group that preceded them, thus producing two daughter nuclei each containing two types of chromosomes. Only one case was found in which all the chromatin material was not included in the two-daughter nuclei.

The homotypic division shows, in the anaphase, many of the univalent chromosomes which, having split in the previous division, lag on the spindle. These go at random to the poles and are included with the divided chromosomes in the formation of the daughter nuclei or lag behind and are left in the cytoplasm to form dwarf nuclei.

The chromosome behavior of the third tetraploid cross, *Triticum spelta* × *T. monococcum*, differs very little from that described for the two preceding crosses. Figure 6, A, shows a cell with 28 univalent chromosomes, while B shows 5 paired and 18 univalent chromosomes. The variation in the number of bivalents observed is within the range of these two illustrations. Many of the univalents split in the first reduction division, and usually all of the chromatin material is in-

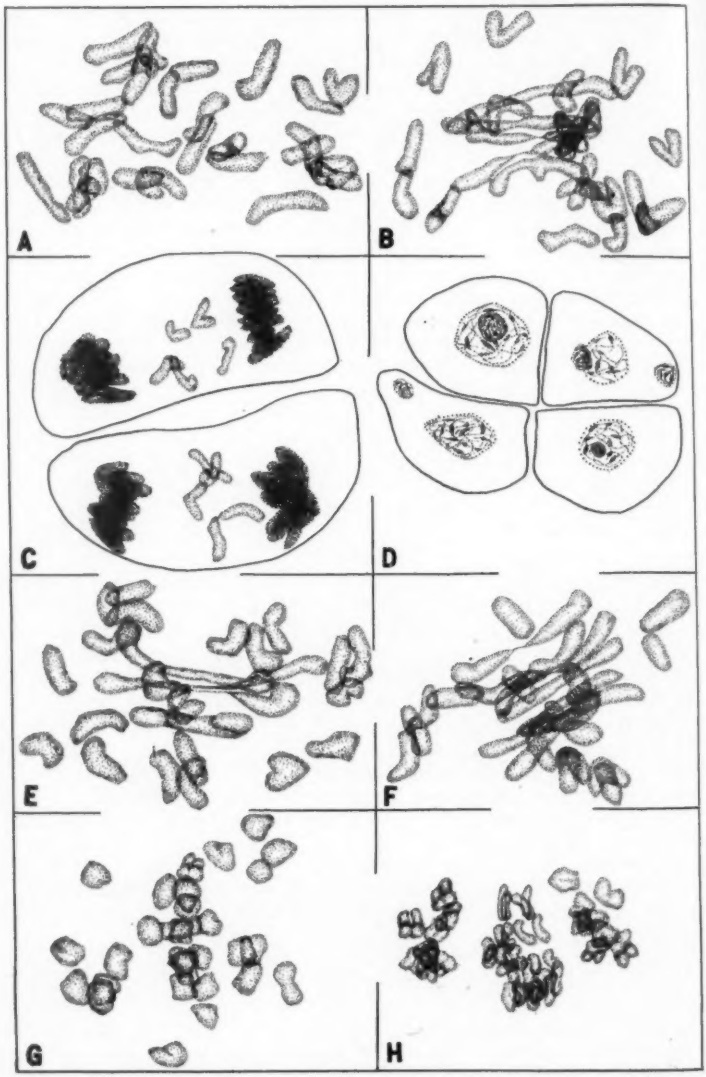


FIGURE 6.—Chromosomes in Triticum × Triticum hybrids. × 2,000: A, Heterotypic prophase in *T. spelta* × *T. monococcum;* B, heterotypic metaphase in *T. spelta* × *T. monococcum;* C, homotypic anaphase in *T. spelta* × *T. monococcum;* D, tetrad in *T. spelta* × *T. monococcum;* E, F, G, three heterotypic metaphases in *T. vulgare* × *T. monococcum;* H, heterotypic anaphase in *T. vulgare* × *T. monococcum*

cluded in the two daughter nuclei. C shows a characteristic homotypic anaphase. At this stage several of the halves of univalents produced in the first division are seen lagging on the spindle. In D is shown a characteristic tetrad, each cell having one or two minor nuclei in addition to the major nucleus.

Characteristic heterotypic metaphases and anaphases in the cross *Triticum vulgare* × *T. monococcum* are shown in Figure 6, E–H. In E are shown 4 bivalent and 20 univalent chromosomes, while in F there are 7 bivalents and 14 univalents. These cells represent about the range of variation observed. In G and H are shown a large number of univalents arranged on the equatorial plate. Later phases show the univalents on the plate splitting longitudinally, the halves joining the earlier divided chromosomes that preceded them to the poles.

The second division is similar to that described for the preceding cross. Tetrads are formed, many individual cells of which are polynucleated.

Melburn and Thompson (*22*) describe the chromosome behavior in the above tetraploid hybrid. They found 0–5 bivalent and 28–18 univalent chromosomes in the early heterotypic phases. Some of the univalents split in the first reduction division, and the halves were seen lagging on the homotypic anaphase spindle in their random passage to the poles. Some chromosomes were extruded in both divisions. The writers find that only very rarely are chromosomes extruded in the first division, but much extrusion of lagging chromosomes may occur in the second division. In agreement with Melburn and Thompson, the writers find that a majority of the pollen tetrads have only four cells, and that many of these cells may be polynucleated.

PENTAPLOID HYBRIDS

No pentaploid hybrids were studied by the writers, but their studies with triploid and tetraploid hybrids afforded an opportunity to compare the meiotic behavior of their hybrids with that observed by others in pentaploid hybrids.

Kihara (*17*, *19*) was the first to investigate the chromosome behavior of Triticum × Triticum hybrids. He found in the early heterotypic phases of pentaploid hybrids 14 bivalent and 7 univalent chromosomes. Many of the bivalent chromosomes were seen to split in the first division, and some of the halves of these split chromosomes were left behind in the cytoplasm in both reduction divisions. Sax (*37*) reported a very similar behavior with the exception that he found practically no chromosomes left free in the cytoplasm during the first division.

The chromosomes of pentaploid hybrids appear to be distributed to the four daughter cells in pollen formation in a manner similar to that described for triploid and tetraploid Triticum × Triticum hybrids.

GENERAL MEIOTIC PHENOMENA IN TRITICUM × TRITICUM HYBRIDS

Plate 2 shows a series of photomicrographs intended to give a somewhat continuous picture of the important meiotic phases in the developing pollen mother cells of Triticum hybrids.

An early heterotypic prophase of *Triticum compactum* × *T. monococcum* in which the individual chromosomes can not be distin-

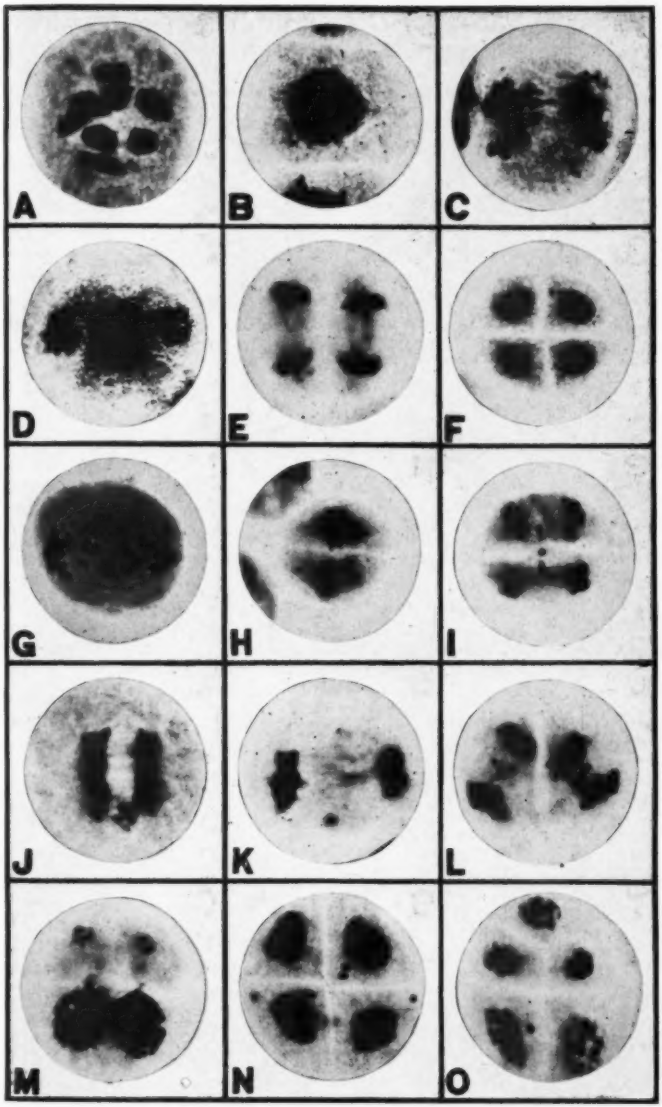
guished is shown in Plate 2, A. B (*T. dicoccoides* × *T. monococcum*) and C (*T. spelta* × *T. monococcum*) show typical heterotypic metaphases. The bivalents and a few univalents are at the equatorial plate, and other univalents are scattered through the spindle region. D, E, and F show heteroptypic anaphases of *T. turgidum* × *T. monococcum*, *T. spelta* × *T. monococcum*, and *T. dicoccoides* × *T. monococcum*, respectively, in which the groups near each pole are made up of halves of bivalent chromosomes and univalents that failed to move to the equatorial region. In D, other univalents have collected on the plate and are in the process of splitting longitudinally. In E, these chromosomes have split and in F they have moved toward the poles. G shows in *T. turgidum* × *T. monococcum* two groups of chromosomes at each pole, a condition frequently seen in the early heterotypic telophase. H (*T. compactum* × *T. monococcum*) and I (*T. turgidum* × *T. monococcum*) show interkinesis. The appearance of pollen mother cells at this stage gives no indication of the chromosome irregularities that characterize the heterotypic metaphase and anaphase. J (*T. vulgare* × *T. monococcum*) shows a homotypic metaphase in which most of the chromosomes are at the plates, but a few univalents that split in the previous division are scattered through the spindles. K (*T. spelta* × *T. monococcum*) and L (*T. dicoccoides* × *T. monococcum*) are typical homotypic anaphases with chromosomes lagging on the spindle. The micronuclei in the cells of the tetrads of *T. polonicum* × *T. monococcum*, shown in M and N, are produced by the chromosomes that were seen lagging on the homotypic anaphase spindle and failed to be included in the four major nuclei. O shows a late homotypic telophase of *T. compactum* × *T. monococcum* in which three instead of two nuclei were produced by the heterotypic division. Such a 3-celled individual will produce a 6-celled tetrad. Tetrads with more than four cells are rare in Triticum × Triticum hybrids.

The cytological work on F_1 interspecific hybrids of Triticum is summarized in Table 3.

CHROMOSOMES IN F_1 HYBRIDS OF TRITICUM × SECALE

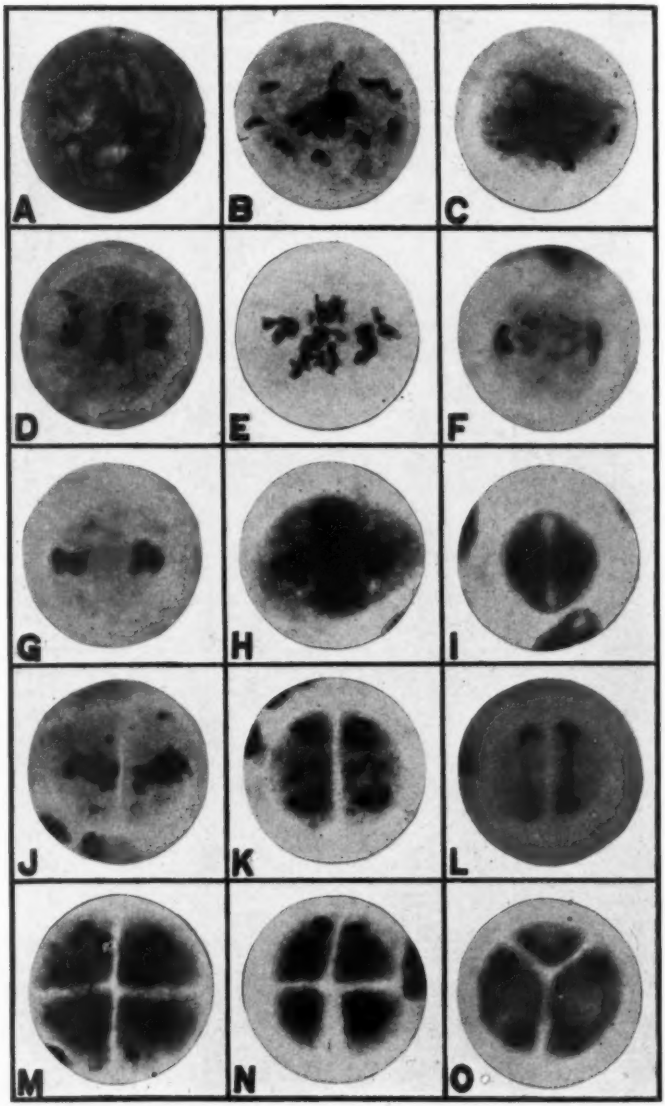
This study includes a triploid and four tetraploid hybrids between the genera Triticum and Secale.

The hybrid *Triticum dicoccoides* × *Secale montanum* shows 21 univalent chromosomes scattered through the spindle in the early metaphase stage. Figure 3, D, shows a typical prophase, while E shows a cell with a majority of the chromosomes on the equatorial plate (the nearest approach found to a true metaphase stage). Those on the plate split and move toward the undivided univalents situated near the poles. It is not unusual to find four nuclei, two of which contain only unsplit univalents while the other two have only split univalents. Other cases were found in which split and unsplit univalents were included in a single nucleus on one side of the cell, while on the other side were two nuclei, one of which contained split and the other unsplit univalent chromosomes. Some cases were observed in which a single nucleus containing both split and unsplit chromosomes was situated on each side of the cell. A more abnormal condition was found in which the univalents on the equatorial plate failed to divide, forming a nucleus in this central position, while those at the poles formed other nuclei. Under such conditions three cells were formed. In cases



PHOTOMICROGRAPHS OF MEIOTIC PHASES IN DEVELOPING POLLEN MOTHER CELLS OF TRITICUM SPECIES AND F_1 AEGILOPS × AEGILOPS AND F_2 AEGILOPS × TRITICUM HYBRIDS. × 875

A, Diakinesis in *T. monococcum* L.; B, heterotypic metaphase in *T. vulgare* Vill.; C, heterotypic anaphase in *T. vulgare;* D, heterotypic telophase in *T. vulgare;* E, homotypic telophase in *T. compactum* Host; F, tetrad in *T. spelta* L.; G, heteroptypic anaphase in *A. triuncialis* × *A. ovata;* H, homotypic metaphase in *A. triuncialis* × *A. ovata;* I, homotypic anaphase in *A. triuncialis* × *A. ovata;* J, heterotypic anaphase in F_2 hybrids of *A. ovata* × *T. dicoccum;* K, heterotypic telophase in F_2 hybrids of *A. ovata* × *T. dicoccum;* L, homotypic anaphase in F_2 hybrids of *A. ovata* × *T. dicoccum;* M, N, O, tetrads in F_2 hybrids of *A. ovata* × *T. dicoccum*



PHOTOMICROGRAPHS OF MEIOTIC PHASES IN THE DEVELOPING POLLEN MOTHER CELLS OF TRITICUM × TRITICUM HYBRIDS. × 875

A, Early heterotypic prophase in *T. compactum* × *T. monococcum;* B, heterotypic metaphase in *T. dicoccoides* × *T. monococcum;* C, heterotypic metaphase in *T. spelta* × *T. monococcum;* D, heterotypic anaphase in *T. turgidum* × *T. monococcum;* E, heterotypic anaphase in *T. spelta* × *T. monococcum;* F, later heterotypic anaphase in *T. dicoccoides* × *T. monococcum;* G, heterotypic telophase in *T. turgidum* × *T. monococcum;* H, interkinesis in *T. compactum* × *T. monococcum;* I, interkinesis in *T. turgidum* × *T. monococcum;* J, homotypic metaphase in *T. vulgare* × *T. monococcum;* K, homotypic anaphase in *T. spelta* × *T. monococcum;* L, late homotypic anaphase in *T. dicoccoides* × *T. monococcum;* M, N, tetrads in *T. polonicum* × *T. monococcum;* O, an unusual homotypic telophase in *T. compactum* × *T. monococcum.*

where all the univalents go at random to the two poles two cells are formed, each containing a nucleus. The nuclei are equal in size if approximately equal numbers of chromosomes go to each pole, but frequently they are quite unequal when a greater number of chromosomes go to one pole than to the other.

Such erratic behavior of chromosomes in the first division give many types of irregularities in the second division and leads to the production of a variety of abnormal tetrads. Single tetrads with four equal-sized cells are rarely observed. The size and number of cells in a tetrad are indicative of the irregularity of distribution of chromosomes in both reduction divisions.

The chromosome behavior in the tetraploid hybrid *Triticum vulgare* × *Secale montanum* differs very little from the triploid hybrid described above. Figure 3, F, shows an early anaphase with 28 univalent chromosomes. In G a bivalent may be seen among the scattered univalents, a condition not usually found.

A third hybrid, *Triticum vulgare* × *Secale cereale*, was studied. Figure 3, H, shows a heterotypic prophase with the 28 chromosomes scattered through the spindle. The chromosome behavior during pollen formation does not seem to differ from that described in the two preceding hybrids except that no bivalent chromosomes were observed in the early heterotypic phases.

The tetraploid hybrid *Triticum spelta* × *Secale montanum* showed as high as three bivalent chromosomes on the heterotypic metaphase plate. With the exception of this slightly greater pairing of the chromosomes, the behavior during the reduction divisions was as abnormal as that found in other Triticum × Secale hybrids.

In Table 4 are listed the various investigators of the chromosomes in Triticum × Secale hybrids. Two of these investigators, namely, Kihara (*19*) and Thompson (*46*), picture very clearly the more critical phases in the reduction divisions in pollen formation. Both investigators found from 0 to 3 bivalent chromosomes in the early phases of the heterotypic division. Table 4 shows that there is no appreciable amount of pairing in any of the Triticum × Secale hybrids studied by the writers and other investigators. Thompson (*46*) found very little tendency for chromosomes to form a true metaphase plate. Kihara (*19*) observed a considerable number of chromosomes going at random to the poles, but also saw collected on the equatorial plate a group of univalents which split, the halves moving to the poles. The writers found many cases similar to those described by Kihara in which a somewhat definite equatorial plate was formed. All of the investigators seem to agree that the first division of Triticum × Secale hybrids frequently produces more than two nuclei, which is a very different condition from that observed in Triticum × Triticum hybrids.

In spite of the irregular behavior in the first division of these bigeneric hybrids, there is a tendency for the second division to function normally. Nuclei containing chromosomes unsplit in the first division divide regularly. The unsplit chromosomes in nuclei containing both split and unsplit chromosomes divide regularly, while the unsplit chromosomes pass at random to the poles or are extruded into the cytoplasm and form micronuclei. The writers' observations show that mother cells that produce more than two nuclei in the first division generally produce tetrads with more than

four cells, whereas if only two nuclei are formed in the first division, normal tetrads result. These cells, however, may have one or more nuclei, depending upon the character of the second division.

TABLE 4.—*Summary of cytological work on F_1 Triticum × Secale hybrids*

Hybrid combination		Chromosome number of parents		Chromosome pairing	Authority	Year
Female	Male	Female	Male			
T. vulgare	S. cereale	8	8		Nakao (*24*)	1911
Do	do	21	7	0-3	Thompson (*46*)	1926
Do	do	21	7	0-3	Kihara (*19*)	1924
Do	do	21	7		Nikolaewa (*28*)	1924
Do	do				Zalensky and Doroshenko (*50*)[a]	1924–25
Do	do	21	7	0	Present writers	
Do	S. montanum	21	7	0-1	do	
T. spelta	do	21	7	0-3	do	
T. dicoccoides	do	14	7	0	do	

[a] No counts or varieties indicated in English summary.

Plate 3 shows in a general way a series of characteristic meiotic phases. These photomicrographs do not attempt to illustrate the innumerable irregularities observed in the developing pollen mother cells, but represent a few of the more typical conditions arranged in a progressive order from the spireme to the early homotypic phases.

A spireme of *Triticum vulgare* × *Secale cereale* and a synoptic phase of *T. spelta* × *S. montanum* are shown in Plate 3. A and B were given very little detailed attention. The writers' studies were confined to the later phases in which the chromosomes were definitely formed and their number and behavior were clearly apparent. C and D show cells of *T. spelta* × *S. montanum* in the heterotypic prophase without any paired chromosomes. In C the chromosomes are scattered on the spindle; in D they are clumped together in the central region. E is a heterotypic anaphase of *T. spelta* × *S. montanum* showing the chromosomes, with one exception, collected in two groups at the poles, without any evidence of a metaphase plate. F shows a cell of *T. spelta* × *S. montanum* with two bivalents and a large number of univalents in the equatorial region and a few univalents at each pole. G shows a late heterotypic anaphase of *T. spelta* × *S. montanum* with a few chromosomes which failed to be included in the two major groups at the poles that have moved to the upper side of the spindle. H, of *T. spelta* × *S. montanum*, shows a cell in the same phase, but in this case a metaphase plate has been formed and the splitting univalents are assembled on the spindle. I and J are heterotypic telophases of *T. spelta* × *S. montanum*. The former shows three almost equal-sized chromatin masses, while the latter shows the formation of three nuclei, two of which are in the early telophase, the other nucleus being advanced to the late telophase. K shows a cell of *T. spelta* × *S. montanum* in which a large mass of undivided chromosomes have collected in the equatorial region and are being cut in two by the division of the cell. L, of *T. spelta* × *S. montanum*, shows a later phase of the phenomenon pictured in K. M and N, of *T. spelta* × *S. montanum*, and O, of *T. dicoccoides* × *S. montanum*, show typical cells produced by the abnormal heterotypic divisions.

CHROMOSOMES IN AEGILOPS SPECIES

A study of the chromosome number in the pollen mother cells of eight species of the genus Aegilops confirms the results of many of the investigators listed in Table 5. These species may be separated into diploid, tetraploid, and hexaploid groups, with seven as the reduced chromosome number in diploid species and multiples of this basic number in polyploid forms.

Aegilops squarrosa, as shown in Figure 7, A, has seven chromosomes at diakinesis. This number agrees with the determinations of Percival (*32*) and Sorokina (*42*). Kihara (*19*), Emme (*8*), Aase and Powers (*1*), and Kagawa (*16*) have found 14 haploid chromosomes in this species. The species examined by the latter investigators may have been *A. ventricosa*. The *A. ventricosa* examined by the writers was received originally under the name of *A. squarrosa*. These two species are morphologically different and may be easily separated.

There seems to be no dispute concerning the chromosome number for *Aegilops speltoides*. Figure 7, B, shows seven chromosomes on the heterotypic metaphase plate, and C shows a late anaphase with seven chromosomes nearing each pole.

Percival (*31*) is the only investigator that has not found 14 as the reduced chromosome number for *Aegilops cylindrica*. Figure 7, D, shows 14 chromosomes of *A. cylindrica* at diakinesis.

An early heterotypic anaphase in a pollen mother cell of *Aegilops ventricosa* is shown in Figure 7, E. Three chromosomes are still undivided, and 11 are seen going to each pole. Six other investigators (Table 5) have found this species to be tetraploid.

Aegilops ovata seems to be represented by a diploid and a tetraploid form, according to the recent studies of Schiemann (*41*). Other investigators (Table 5) picture 14 as the reduced chromosome number for this species. Figure 7, F, shows 14 chromosomes on each homotypic metaphase plate of *A. ovata*.

There seems to be no disagreement in assigning *Aegilops triuncialis* to the tetraploid group. Figure 7, G, shows a late heterotypic anaphase with 14 chromosomes at each pole. The plants for this study were grown from seed received as *A. triticoides*, but a careful study of the plants showed them to be *A. triuncialis*.

Aegilops triaristata is also a tetraploid species. Figure 7, H, shows the 14 chromosomes on the heterotypic metaphase plate. The same chromosome number for this species has recently been reported by Sorokina (*42*).

The chromosome number of *Aegilops crassa* seems to differ in different forms. Percival (*32*) found one form that is tetraploid and another that is hexaploid. Figure 7, I, shows a late heterotypic anaphase with 21 chromosomes at each pole. This number places the form studied by the writers in the hexaploid group. Emme (*8*), and more recently Sorokina (*42*), found 21 as the chromosome number in their material of this species.

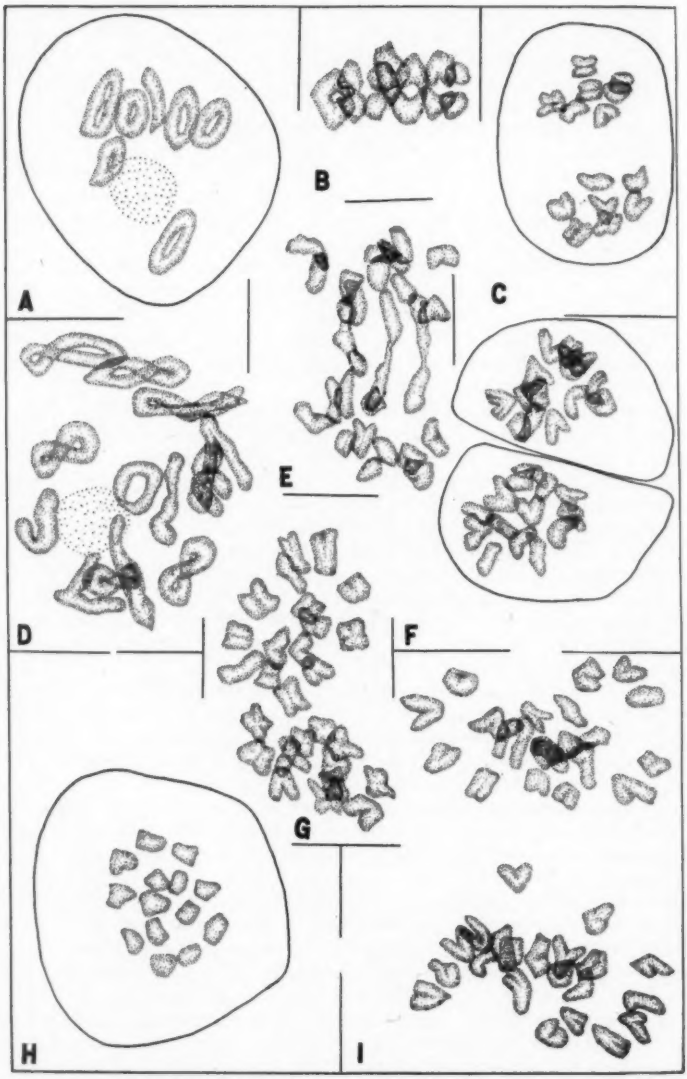


FIGURE 7.—Chromosomes in Aegilops species. × 2,000: A, Diakinesis in *A. squarrosa* L.; B, heterotypic metaphase in *A. speltoides* Tausch.; C, heterotypic anaphase in *A. speltoides*; D, diakinesis in *A. cylindrica* Host.; E, early heterotypic anaphase in *A. ventricosa* Tausch.; F, homotypic metaphase in *A. ovata* L.; heterotypic anaphase in *A. triuncialis* L.; H, heterotypic metaphase in *A. triaristata* Willd.; I, heterotypic anaphase in *A. crassa* Boiss.

TABLE 5.—*Chromosome numbers of some Aegilops species as determined by the investigators named*

[Diploid chromosome numbers in figure columns are shown in italics; all other numbers are haploid chromosomes]

Investigator	A. ovata	A. triaristata	A. triuncialis	A. triticoides [a]	A. biuncialis	A. cylindrica	A. squarrosa	A. caudata	A. comosa	A. uniaristata	A. ventricosa	A. speltoides	A. aucheri	A. bicornis	A. crassa	A. longissima	A. turcomanica	A. variabilis
Bally, 1912 (*3*) 1919 (*4*)	16																	
Percival, 1923 (*31*), 1926 (*32*)	14		14			7	7				14	7			[b] 14			
Kihara, 1924 (*19*)	*28*, 14			[a] *28*			[c] *28*				*28*							
Sax and Sax, 1924 (*40*)	14					14												
Emme, 1924 (*8*)			*28*			*28*	[c] *28*				*28*				*28*			
Aase and Powers, 1926 (*1*)	14		14			14	[c] 14											
Gaines and Aase, 1926 (*11*)						14												
Tschermak and Bleier, 1926 (*47*)	14																	
Bleier, 1928 (*7*)						14		7			14							
Sorokina, 1928 (*42*)	14	14	14		14	14	7	7	7		14	7	7	7	21	7	21	14
Schiemann, 1928 (*41*)	[d] 14		14			*28*		*14*	*14*	*14*	14	7						
Kagawa, 1928 (*16*)	*28*, 14		14			*28*, 14	[c] 14					*14*, 7						
Present writers	14	14	14			14	7				14	7			21			

[a] *A. triticoides* is not considered a true species; the form examined by Kihara was probably *A. triuncialis*.
[b] Another form of this species was examined with 21 haploid chromosomes.
[c] It is probable that these investigators have confused *A. ventricosa* with *A. squarrosa*.
[d] Another form of this species was examined with 7 haploid and 14 diploid chromosomes.

CHROMOSOMES IN F_1 HYBRIDS OF AEGILOPS SPECIES

The writers had cytological material available of one F_1 hybrid of Aegilops species. Heterotypic metaphases of pollen mother cells of *A. triuncialis* × *A. ovata* show considerable pairing of chromosomes. The number frequently seen was 7, but lower numbers were common. After the bivalents divide, many of the univalents move to the plate and split longitudinally. (Pl. 1, G.) Heterotypic telophases show that all the chromosomes are included in the two daughter nuclei. The second reduction division shows many of the univalents that split in the first division moving tardily to the metaphase plate. (Pl. 1, H.) These univalents lag on the anaphase spindle, as shown in Plate 1, I. The lagging chromosomes pass at random to the two poles or remain behind and are left outside the major nuclei. There usually are only four pollen grains from a pollen mother cell. Some cells of the tetrad, however, may contain, in addition to the major nucleus, one or more minor nuclei. Only a limited number of Aegilops × Aegilops hybrids have been studied, as shown in Table 6.

TABLE 6.—*Summary of cytological work on F_1 Aegilops × Aegilops hybrids*

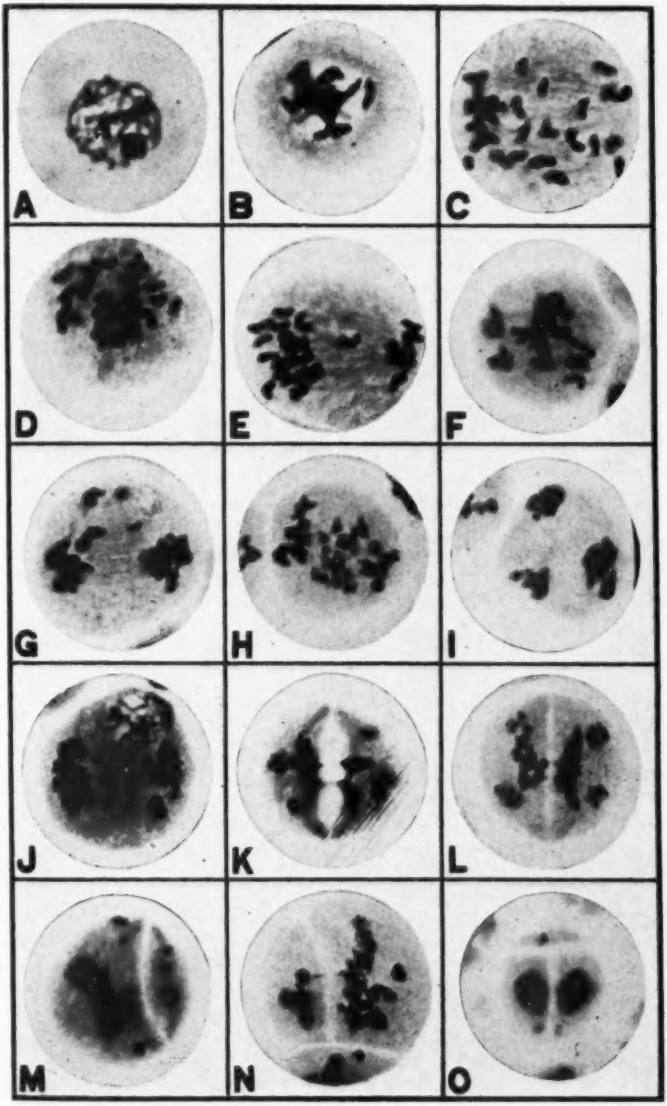
Hybrid combination		Chromosome number		Chromosome pairing	Authority	Year
Female	Male	Female	Male			
A. ovata	A. caudata	14	7	7–10	Bleier (7)	1928
Do	A. triuncialis	14	14	0–7	Present writers	

Bleier (7) described a very similar condition for the triploid hybrid *Aegilops ovata* × *A. caudata*. Heterotypic prophases show from 7 to 10 bivalent chromosomes and a few univalents. The latter split in the first division but were included in the two nuclei formed. Tetrads observed by him usually were 4-celled.

CHROMOSOMES IN HYBRIDS OF AEGILOPS × TRITICUM

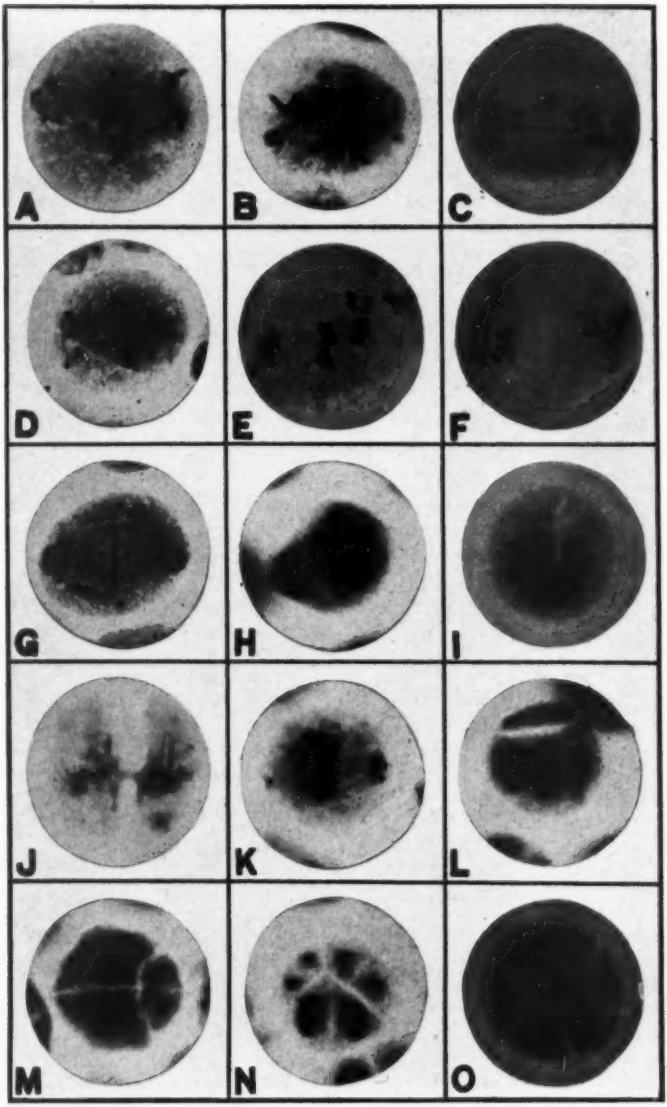
The writers had available cytological material for a study of the meiotic phases of the developing pollen mother cells of two tetraploid, five pentaploid, and three hexaploid Aegilops × Triticum hybrids. Several F_1 plants of the cross *A. crassa* × *T. monococcum* were produced but perished while quite young, thereby failing to provide material for cytological analysis.

The cross *Aegilops cylindrica* × *Triticum turgidum* furnished a good series of the various meiotic phases. Figure 4, F and G, shows two characteristic prophases. F shows two bivalent and 24 univalent chromosomes, and G shows only univalent chromosomes. Three paired chromosomes were the largest number observed, and in a large percentage of the cells all the chromosomes were unpaired. Ordinarily many chromosomes collect at the metaphase plate and a few remain at either pole. The normal procedure is for chromosomes at the equatorial plate region to divide longitudinally and the halves move to the poles. In some cases, however, the central mass of chromosomes assumes a condition typical of the telophase stage. (Pl. 4, H.) A circle of chromatin material is formed at the plate region, which is divided into two parts by the formation of the cell walls. Occasionally,



PHOTOMICROGRAPHS OF MEIOTIC PHASES OF DEVELOPING POLLEN MOTHER CELLS IN TRITICUM × SECALE F_1 HYBRIDS. × 875

A, Spireme in *T. vulgare* × *S. cereale;* B, synapsis in *T. spelta* × *S. montanum;* C, D, F, heterotypic prophases in *T. spelta* × *S. montanum;* E, G, H, heterotypic anaphases in *T. spelta* × *S. montanum;* I, J, heterotypic telophases in *T. spelta* × *S. montanum;* K, L, N, interkinesis in *T. spelta* × *S. montanum;* M, N, early homotypic metaphase in *T. spelta* × *S. montanum;* O, interkinesis in *T. dicoccoides* × *S. montanum.*



PHOTOMICROGRAPHS OF DEVELOPING POLLEN MOTHER CELLS IN F_1 AEGILOPS × TRITICUM HYBRIDS. × 875

A, B, D, Heterotypic metaphases in *A. crassa* × *T. spelta;* C, E, F, heterotypic anaphases in *A. crassa* × *T. dicoccum;* G, heterotypic telophase in *A. crassa* × *T. spelta;* H, K, heterotypic metaphases in *A. cylindrica* × *T. turgidum;* I, cell division in *A. crassa* × *T. durum;* J, cell division in *A. crassa* × *T. turgidum;* L, M, homotypic telophases in *A. cylindrica* × *T. turgidum;* N, tetrad (?) in *A. crassa* × *T. polonicum;* O, tetrad (?) in *A. crassa* × *T. dicoccum*

however, at the time of the division of a cell, the dividing wall, instead of passing directly through the center and forming two equal-sized daughter cells, may circumflect the mass, leaving all of the chromatin material in one cell, while the other cell will be devoid of this substance. At the same time other cell walls may appear and cut off the nuclei formed by the chromosomes at the poles if some have remained there instead of joining the central mass. The cells produced in the heterotypic division in most cases divide regularly in the homotypic division, and most of the figures show no lagging of chromosomes on the spindle. This phenomenon suggests that univalent chromosomes rarely split and that the chromatin mass divides amitotically in the first division. The number of cells of the pollen tetrads vary, depending on the character of the heterotypic division.

The second tetraploid hybrid, *Aegilops cylindrica* × *Triticum polonicum*, shows during meiosis no noticeable differences from that described for the previous hybrid. Figure 4, H, shows the chromosomes from a pollen mother cell with three paired chromosomes, while I shows only univalents scattered through the spindle.

In the pentaploid hybrid *Aegilops crassa* × *Triticum turgidum* the chromosomes are scattered through the spindle region in the prophase. Frequently these chromosomes all appear as univalents, but sometimes as high as four bivalents were observed, as shown in Figure 4, B. This amount of pairing is slightly more than that noted in any of the tetraploid hybrids. The chromosome behavior in the later stages is similar in every detail to that described for the two previous hybrids. The tetrads resulting from such meiotic behavior are irregular, and the cells vary both in number and size.

The pentaploid hybrid *Aegilops crassa* × *Triticum polonicum* shows no outstanding differences in chromosome behavior from the preceding pentaploid hybrid. Figure 8, C, is a prophase stage showing 35 univalent chromosomes, and J is a similar phase showing 3 bivalent chromosomes. D is a later phase showing univalent chromosomes at the two poles and a large group at the plate. All of the latter chromosomes show some indications of undergoing a longitudinal division. A few cases were noticed in which those at the equatorial plate have split and moved to join the groups at the poles. The more usual condition is shown in E, with one chromosome remaining at the pole, while the others have moved to the equatorial region, where they have taken on the appearance of chromosomes in a telophase stage. In the process of cell division as shown in E there may be seen at one end a cell containing one chromosome and a second wall in the equatorial region in the process of dividing the chromatin mass into two large cells with equal quantities of chromatin material. In F and G are shown cells resulting from irregular heterotypic divisions. F shows one cell with a single split chromosome and two cells with slightly unequal amounts of chromatin material. H shows a 6-celled tetrad resulting from such abnormal chromosome behavior as shown in F.

Hybrids of *Aegilops crassa* × *Triticum durum* show in typical prophases (fig. 9, H) 35 unpaired chromosomes, while only a few cases with bivalent chromosomes were seen. In Figure 9, D–G, are shown the usual method of chromosome and cell division, a true heterotypic division being rarely observed.

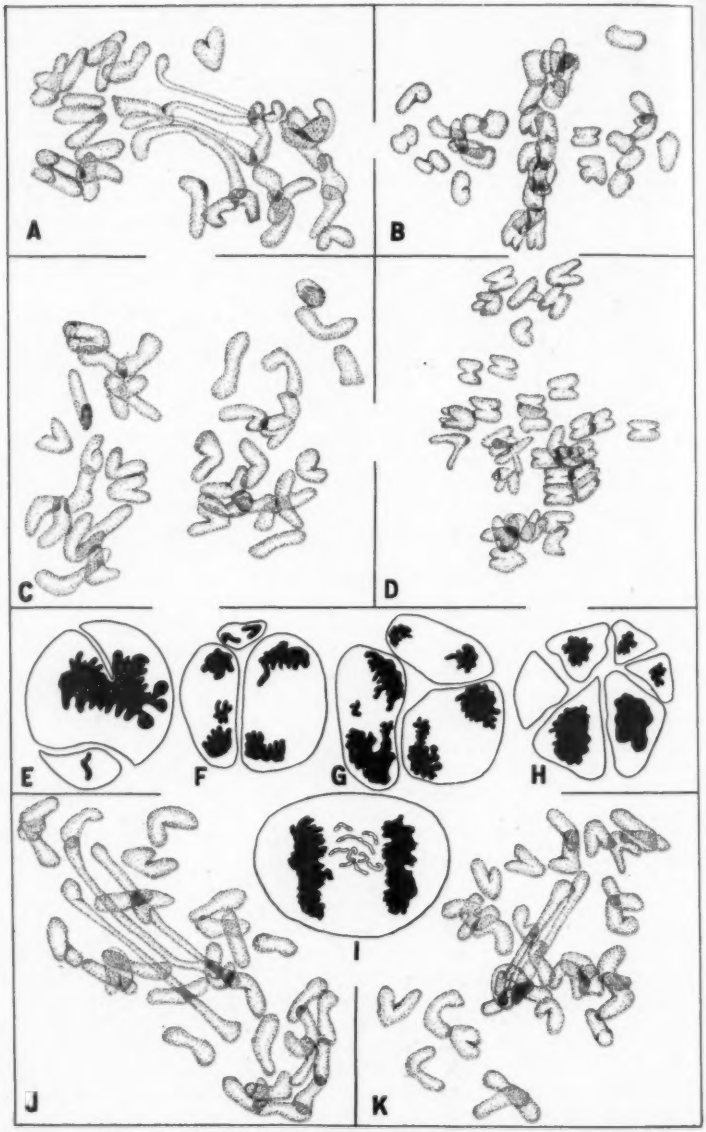


FIGURE 8.—Chromosomes in Aegilops × Triticum hybrids: A, Heterotypic metaphase in *A. crassa* × *T. dicoccoides;* B, heterotypic anaphase in *A. crassa* × *T. dicoccoides;* C, heterotypic prophase in *A. crassa* × *T. polonicum;* D, heterotypic anaphase in *A. crassa* × *T. polonicum;* E, interkinesis in *A. crassa* × *T. polonicum;* F, late homotypic anaphase in *A. crassa* × *T. polonicum;* G, late homotypic anaphase in *A.* crassa × *T. polonicum;* H, tetrad in *A. crassa* × *T. polonicum;* I, heterotypic anaphase of F_2 of *A. ovata* × *T. dicoccum;* J, heterotypic metaphase in *A. crassa* × *T. polonicum;* K, heterotypic metaphase in *A. crassa* × *T. dicoccum.* A–D, J, × 2000; E–I, × 875

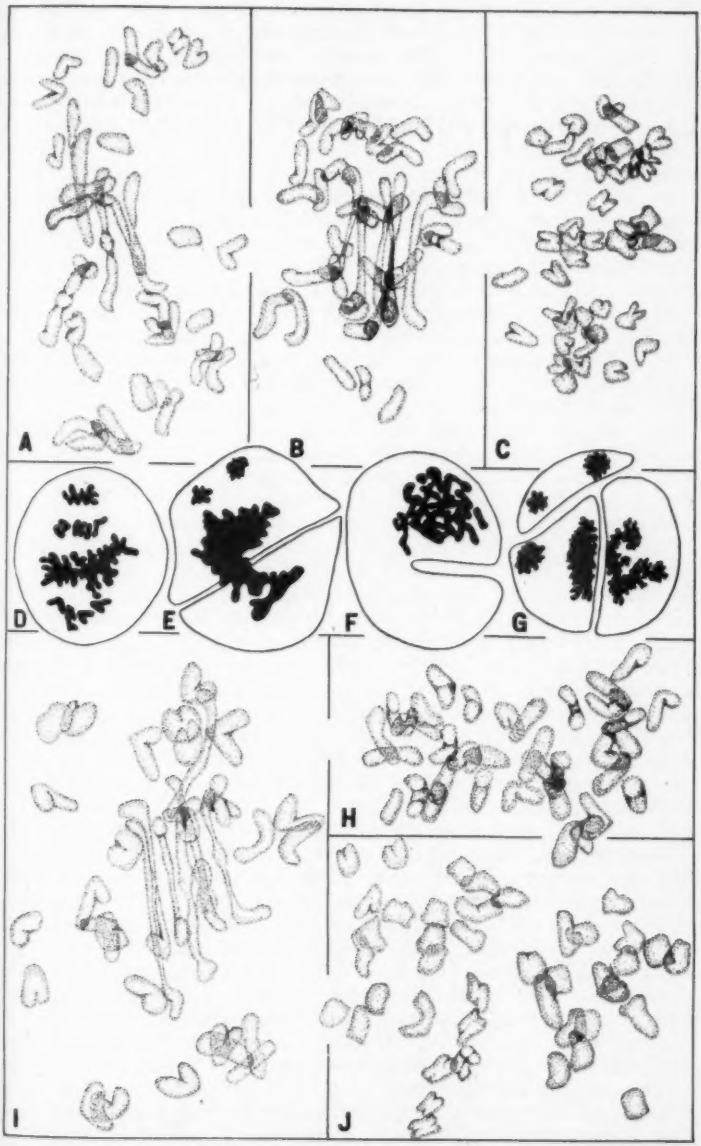


FIGURE 9.—Chromosomes in Aegilops × Triticum hybrids: A, Heterotypic metaphase in *A. crassa* × *T. spelta;* B, heterotypic metaphase in *A. crassa* × *T. vulgare;* C, heterotypic anaphase in *A. crassa* × *T. vulgare;* D, heterotypic anaphase in *A. crassa* × *T. durum;* E, F, G, interkinesis in *A. crassa* × *T. durum;* H, heterotypic prophase in *A. crassa* × *T. durum;* I, heterotypic metaphase in *A. crassa* × *T. compactum;* J, heterotypic anaphase in *A. crassa* × *T. compactum.* A–F, H–J, × 2000; D–G, × 875

Figure 9, D, shows the distribution of the chromosomes into four groups, while E is a later phase showing the cell wall cutting through the major centrally located chromosome mass. In F, which is unusual, the cell wall, instead of passing through the chromosomes, passes to one side, forming one cell without a nucleus. In G a later phase is shown. It is difficult to say definitely what phase it represents, but it appears to be a late heterotypic phase, derived from a cell which passed through a stage similar to that shown in E. If so, one expects each chromosome mass to split equally in the homotypic division and produce either 6 pollen grains, 4 of which will be binucleated; 8 pollen grains, 2 of which will be binucleated; or 10 mononucleated pollen grains.

The fourth pentaploid hybrid, *Aegilops crassa* × *Triticum dicoccum*, appears in most reduction phases to be identical with the preceding hybrids. There seems, however, to be a somewhat greater tendency for the chromosomes to pair. Figure 4, A, and Figure 8, K, show heterotypic metaphases with two bivalent chromosomes. Some cells were observed with as many as six pairs of chromosomes.

The description of the meiotic phases in the pentaploid hybrid *Aegilops crassa* × *Triticum dicoccoides* was purposely reserved for consideration until the last. In it was found a chromosome behavior quite distinct from that described for the preceding hybrids and similar to that described for Triticum × Triticum hybrids.

The number of paired chromosomes usually seen was four. Figure 8, A, shows a typical distribution of the chromosomes at the early metaphase. B is a typical picture of a later phase, showing the divided halves of bivalents with a few univalents at each pole and a large number of univalents assembled on the equatorial plate preparing to split longitudinally, after which operation they separate and join the two groups at the poles. At interkinesis, therefore, there are produced two equal-sized mononucleated cells, each nucleus containing two types of chromosomes. The homotypic division is irregular, due to the presence of the split univalents. The homotypic anaphase shows many of them on the spindle, going at random to the poles, and frequently a few, too late to be included in the daughter nuclei, are left in the cytoplasm to form micronuclei.

Pollen tetrads, with rare exceptions, are four celled. Each cell, however, may have micronuclei in addition to the major nucleus. A count of 43 cells showed 35 with one nucleus only, 6 with a major and a minor nucleus, and 1 with a major and two minor nuclei.

It is very apparent that the behavior of the chromosomes in this Aegilops × Triticum hybrid is similar in every respect to that earlier described for Triticum × Triticum hybrids. Such a chromosome behavior during the meiotic phases suggests that *T. dicoccoides*, the so-called "wild wheat" of Palestine, shows somewhat the same cytological relation to *A. crassa* as *T. monococcum* shows in relation to other species of Triticum.

The hexaploid hybrid *Aegilops crassa* × *Triticum compactum* shows in the early heterotypic phases as high as 7 paired chromosomes. Figure 9, I, shows a cell with 7 dividing bivalent chromosomes and 28 univalents. J shows a later phase with two groups approaching the poles and a few univalents on the equatorial plate. There seems to be a greater tendency in this hybrid than in the earlier described Aegilops × Triticum hybrids for a normal division of the chromo-

somes into two equal masses and the production of two cells in the heterotypic division. Irregularities that produce three or even more cells are abundant, however, and the general chromosome behavior is that described as typical for hybrids between these two genera. The chromosomes in the two to several nuclei divide equally in the homotypic division, giving tetrads with varying numbers of pollen cells.

In the hybrid *Aegilops crassa* × *Triticum vulgare* the condition is very similar to that described in the previous hexaploid hybrid. Four to seven bivalents are most frequent. Figure 9, B, shows a typical early metaphase, while C shows a later phase very similar to that in J of the *A. crassa* × *T. compactum* cross.

Figure 9, A, shows a typical cell of the hybrid *A. crassa* × *T. spelta* with 4 bivalents and 34 univalents.

Hexaploid hybrids in general show more chromosomes pairing and a more normal distribution of chromosomes than are found in tetraploid or pentaploid Triticum × Aegilops hybrids. Tetrad formation, however, is more irregular than that found in the exceptional hybrid *A. crassa* × *T. dicoccoides*. The chromosome behavior in hybrids of Aegilops × Triticum is similar, but is strikingly different from that found in Aegilops × Aegilops and Triticum × Triticum hybrids.

Plate 4 has been prepared to show by means of a series of photomicrographs the developing pollen mother cells of Aegilops × Triticum hybrids.

Plate 4, A and B, are heterotypic metaphases of *Aegilops crassa* × *Triticum spelta* in which some of the chromosomes are in the equatorial region and others are scattered through the spindle. In B two bivalents are shown among the univalents in the group at the plate. C shows a heterotypic anaphase of *A. crassa* × *T. dicoccum* with two groups of chromosomes at each pole and a dividing bivalent stretched between them. D shows a cell of *A. crassa* × *T. spelta* with two groups of chromosomes at the poles and a third group of univalents on the equatorial plate. E shows a later phase of a cell of *A. crassa* × *T. dicoccum* in which the split univalents are moving toward the chromosomes at the poles, and F shows these split univalents very close to the two groups of chromosomes that preceded them to the poles. G shows a telophase of *A. crassa* × *T. spelta* in which the chromosomes, with the exception of two showing faintly on the lower edge of the spindle, are included in two major chromatin masses. H–K show a more abnormal heterotypic division phenomenon. In H all the chromosomes in a cell of *A. cylindrica* × *T. turgidum* are shown in a central position, but instead of undergoing division they have reverted to a condition that is characteristic of chromosomes in the early telophase. K is a cell of *A. cylindrica* × *T. turgidum* in the same phase, but shows in addition to the chromatin mass at the center a few chromosomes at each pole. I shows in *A. crassa* × *T. durum* a centrally located chromatin mass being cut in two by the division of the cell in the formation of a dyad. J shows a later phase of *A. crassa* × *T. turgidum* with a chromatin strand still bridging the gap between the two cells. L and M show homotypic anaphases of typical pollen mother cells of *A. cylindrica* × *T. turgidum*. In the former a regular division of the chromosomes of the two unequal-sized nuclei takes place, while in the latter a regular division of

the three nuclei occur. N, of *A. crassa* × *T. polonicum*, and O, of *A. crassa* × *T. dicoccum*, show pollen tetrads with 6 and 7 cells, respectively.

Table 7 lists the investigators who have made cytological studies of the chromosome behavior in developing pollen mother cells of Aegilops × Triticum hybrids.

TABLE 7.—*Summary of cytological work on F_1 Aegilops × Triticum hybrids*

Hybrid combination		Chromosome number		Chromosome pairing	Authority	Year
Female	Male	Female	Male			
A. ovata	T. monococcum	14	7	0–5	Bleier (7)	1928
Do	T. dicoccum	14	14	0–7	Percival (32)	1926
Do	do	14	14	0	Sax (39)	1928
Do	T. vulgare	14	21	0–3	Bleier (7)	1928
Do	do	14	21	0–few	Percival (32)	1926
A. cylindrica	T. durum	14	14	0	Bleier (7)	1928
Do	T. turgidum	14	14	0	Gaines and Aase (11)	1926
Do	do	14	14	0–3	Present writers	
Do	T. polonicum	14	14	0–3	do	
Do	T. vulgare	14	21	5–7	Sax and Sax (40)	1924
Do	do	14	21	7	Kagawa (16)	1928
Do	T. spelta	14	21	7	Bleier (7)	1928
A. crassa	T. dicoccoides	21	14	0–5	Present writers	
Do	T. dicoccum	21	14	0–6	do	
Do	T. durum	21	14	0–3	do	
Do	T. turgidum	21	14	0–4	do	
Do	T. polonicum	21	14	0–4	do	
Do	T. compactum	21	21	0–7	do	
Do	T. vulgare	21	21	0–7	do	
Do	T. spelta	21	21	0–6	do	
T. durum	A. ovata	14	14		Kagawa (16)	1928
T. vulgare	do	8	16		Bally (4)	1919
Do	do	21	14		Emme (8)	1924
Do	A. cylindrica	21	14	7	Gaines and Aase (11)	1926
Do	do	21	14	7	Kagawa (16)	1928

Bally (*4*) studied the meiotic phases in the pentaploid hybrid *Triticum vulgare* × *Aegilops ovata*. His study was the first of its kind, and though his chromosome counts were not accurate, as has since been proved by other investigators, his observations on the chromosome behavior give a clear general picture of the irregular chromosome distribution in both the heterotypic and homotypic divisions. The irregular tetrads shown by him are typical of Aegilops × Triticum hybrids. The reciprocal cross (*A. ovata* × *T. vulgare*) has been studied more recently by Percival (*32*) and Bleier (*7*). The number of paired chromosomes has been found by them to be small, and the tetrad formation generally irregular.

Bleier (*7*) was the first to study triploid Aegilops × Triticum hybrids. In the F_1 of an *A. ovata* × *T. monococcum* hybrid 21 univalents usually are seen, but a few cases show as many as 5 bivalent chromosomes.

Both Percival (*32*) and Sax (*39*) have studied the chromosome behavior in *Aegilops ovata* × *T. dicoccum*. Percival finds little, if any, pairing of chromosomes in the heterotypic prophase; Sax finds no pairing. Both, however, find tetrad formation very irregular, giving microspores of various sizes and in varying numbers. Gaines and Aase (*11*) observed no chromosome pairing in the F_1 hybrid of *A. cylindrica* × *T. turgidum*. Bleier (*7*) has described some of the meiotic phases in the tetraploid hybrids of *A. cylindrica* × *T. durum*.

No pairing was observed, and all univalent chromosomes go at random to the poles without splitting.

Sax and Sax (*40*), Gaines and Aase (*11*), and more recently Kagawa (*16*) have studied the meiotic phases in pollen mother cells of F_1 hybrids between *Triticum vulgare* and *Aegilops cylindrica*. All of the investigators found 7 bivalent and 21 univalent chromosomes in the heterotypic phases. Kagawa found that the univalents sometimes split in the heterotypic division, these monads being distributed at random to the poles in the homotypic division or left in the cytoplasm. In another pentaploid hybrid, *A. cylindrica* × *T. spelta*, Bleier (*7*) found 7 paired chromosomes in the heterotypic prophase and no indication of univalents splitting in the first division.

Table 7 has been prepared to show the amount of chromosome pairing observed by the writers and other investigators in the early heterotypic phases of Aegilops × Triticum hybrids. It is apparent that seven paired chromosomes is the maximum number found. In most cases, however, the number of paired chromosomes is variable and seldom is as high as seven.

In conjunction with the cytological investigations with F_1 hybrids herein discussed, four F_3 self-pollinated plants of the cross *Aegilops ovata* × *Triticum dicoccum* were studied. In all of these F_3 plants the chromosome number exceeded that of the F_1, but in none was there any indication of a true doubling like that found by Tschermak and Bleier (*47*) in F_5 and F_6 hybrids of *A. ovata* × *T. dicoccoides* and *A. ovata* × *T. durum*.

In the F_3 material studied by the writers, two plants from the same F_1 plant line had 26 and 27 haploid chromosomes, respectively. Another plant from a different F_1 plant line had 25 haploid chromosomes in the pollen mother cells. One other plant was studied in which no chromosome counts were established but in the homotypic division of which from 5 to 6 lagging chromosomes were observed. In the 26-chromosome plant one cell had 26 pairs only, one cell had 25 pairs and 2 splitting univalents, another cell had 22 pairs and 8 splitting univalents, while still another cell had 19 pairs and 14 splitting univalents. In the 25-chromosome plant one cell had 25 pairs only, and one cell had 22 pairs, 3 splitting univalents, and 3 unsplit univalents toward one pole. In each of two other cells 5 univalents were observed splitting, the pairs not being counted. In the 27-chromosome plant 3 cells were observed each with 26 pairs and 2 splitting univalents.

Hybrids between Aegilops and Triticum have been obtained and the successive generations continued for many years by several investigators. Practically all of the hybrids obtained, however, have been subjected to cross-pollination with wheat. Apparently no carefully controlled self-pollination experiments were conducted with F_1 hybrids of such crosses. In order to determine the extent of self-fertility in Aegilops × Triticum hybrids several F_1 plants of the hybrid *A. ovata* × *T. dicoccum* were inclosed in glassine bags during the pollination period by the junior writer. A small percentage of seeds was obtained under these conditions. During the subsequent generations the plants were again self-pollinated under controlled conditions. The cytological studies on the F_3 hybrids of the cross *A. ovata* × *T. dicoccum* have been made from such controlled self-pollinated material.

In 1926 Tschermak and Bleier (*47*) made cytological studies of fertile F_5 F_6 plants of the hybrids *Aegilops ovata* × *Triticum dicoccoides* and *A. ovata* × *T. durum*. Twenty-eight haploid chromosomes were observed in the plants of these hybrids. This doubling was attended by a regular reduction division in the pollen mother cells unlike that found in hybrids. The somatic cells were observed to have 56 chromosomes. In addition, however, to the regular cytological phenomena Tschermak and Bleier (*47*) frequently observed small strongly stained bodies in the cytoplasm of the pollen tetrad cells which they indicated might be extruded chromosomes. When an F_5 F_6 hybrid of the first cross (*A. ovata* × *T. dicoccoides*) was crossed with a hybrid of the second cross (*A. ovata* × *T. durum*), the reduced chromosome number was 28 and the reduction divisions were quite normal. In further investigations Bleier (*7*) backcrossed *A. ovata* with the fertile F_5 F_6 hybrids of *A. ovata* × *T. dicoccoides* and *A. ovata* × *T. durum*. These new F_1 hybrids produced pollen mother cells with 14 bivalent and 14 univalent chromosomes, thus indicating that the male gametes used contained 28 chromosomes.

In the cytological studies by Sax (*39*) of six plants resulting from a cross of the F_1 *A. ovata* × *T. dicoccum* hybrid with the *T. dicoccum* parents, 28 chromosomes were established as the complement of the egg cells in the F_1.

Though a tendency toward doubling of chromosomes was observed by the writers in F_3 hybrid material of a cross between *Aegilops ovata* and *Triticum dicoccum*, the individual plants studied showed from 1 to 3 chromosomes less than a true double number 28. It was characteristic of these plants to form tetrads whose individual cells contain one or more micronuclei besides a major nucleus. Phenomena of this kind appear generally to be the result of irregular behavior of chromosomes in the meiotic phases preceding the formation of tetrads and suggest the presence of nonhomologous chromosomes. It seems quite probable that what appeared to Tschermak and Bleier as strongly stained bodies in the cytoplasm of the cells of the tetrads in their hybrids may have been similar to the micronuclei or extruded chromosomes observed by the writers in their material.

Figure 4, C, shows 26 chromosomes at each pole in the heterotypic metaphase of one of the F_3 plants referred to above. The chromosome behavior in all of the above F_3 hybrids studied was more or less irregular, but was more regular than in any of the F_1 Aegilops × Triticum hybrids previously described. The phenomena generally observed indicate that a large number of chromosomes paired and formed a fairly regular metaphase plate, but some unpaired chromosomes were usually present. Most of the univalents usually split in the heterotypic division (fig. 8, I), and the halves occasionally failed to be included in the major nuclei. The more common procedure, however, is for the univalents to split, the halves going to separate poles, where they are included in the nuclear mass composed of the members of paired chromosomes. The homotypic division often showed halves of univalents lagging on the spindle, some of which failed to be included in the major nuclei but formed one to several micronuclei.

Typical phases of the pollen mother-cell development are illustrated in six photomicrographs shown in Plate 1. A heterotypic anaphase is shown in J, in which practically all chromosomes have

paired and divided. K shows a heterotypic telophase with an extruded chromosome at the lower side of the spindle. L shows a typical homotypic anaphase with several chromosomes lagging on the spindle. Three quite different tetrads are shown in M–O. The first is a tetrad in which the nuclei of two cells are much larger than those of the other two. The second shows micronuclei in three of the cells of the tetrad. The third is a pentad, in which four cells are mononucleated and one cell has a micronucleus in addition to the major nucleus.

DISCUSSION

The chromosome number and general meiotic behavior of the various species of the genera Triticum, Secale, and Aegilops examined by the writers were similar with few exceptions, to those observed by other investigators. The lack of agreement in the chromosome counts of certain species is doubtless due to an improper identification of the species. All of the Triticum × Triticum hybrids studied had a diploid species (*T. monococcum*) as the pollen parent, the female parent being either a tetraploid or a hexaploid species. In such hybrids, which combine species with different chromosome numbers, both paired and unpaired chromosomes were observed. In all of the crosses, however, the amount of chromosome pairing varied from 0 to 7 and showed no indication that a definite number of paired chromosomes was characteristic in any of the hybrids studied.

The unpaired chromosomes were erratic in their behavior, splitting either in the first or in the second reduction division. These split univalent chromosomes generally are included in the two daughter nuclei formed from the first division, but in their random movement to the poles in the second division they frequently lag behind on the spindle and fail to be included in the four major nuclei. These extruded chromosomes form micronuclei, and consequently many cells of the tetrads are polynucleated.

These results are not altogether corroborated by the work of earlier investigators. Thompson (*45*) found a chromosome situation in a triploid Triticum hybrid corresponding quite closely to that herein described. The chromosome behavior of pentaploid hybrids described by Kihara (*17*, *19*) and Melburn and Thompson (*22*) is different from that found in triploid and tetraploid hybrids studied by the writers. Those investigators found chromosomes left in the cytoplasm at the conclusion of both the heterotypic and the homotypic divisions. The writers find this phenomenon very characteristic of the homotypic division but very rare in the heterotypic division. Sax (*37*) described a chromosome behavior of some F_1 Triticum × Triticum hybrids that differs quite noticeably from the studies of more recent investigators, but there seems to be quite general agreement in the character of the tetrads. Triticum × Triticum hybrids, with few exceptions, produce 4-celled tetrads; each cell, however, may have one or more minor nuclei in addition to the major nucleus. In hybrids between species of the emmer group and species of the *vulgare* group a constant number of 14 pairs of chromosomes has been observed by other investigators. Such variable pairing as is indicated in the tetraploid hybrids studied by the writers suggests a more distant relationship between the einkorn group and both the emmer and *vulgare* groups than that existing between the emmer and *vulgare* groups.

The chromosome behavior in Aegilops × Triticum hybrids, with one exception, is similar; i. e., individual differences are apparent but these are often insignificant. The amount of pairing of chromosomes varies from 0 to 7. This variability agrees with the results of recent investigators. Sax and Sax (*40*) and Gaines and Aase (*11*), however, find 7 paired and 14 unpaired chromosomes the general rule in the early heterotypic phases of an *A. cylindrica* × *T. vulgare* hybrid. Only a few cases have been reported with such a definite number of pairs. Crosses of *A. cylindrica* × *T. vulgare* and *A. cylindrica* × *T. spelta* seem to come the nearest to having 7 definitely paired chromosomes, but even in these two crosses lower numbers are reported. The writers' studies of Aegilops × Triticum hybrids show the number of paired chromosomes to be small and variable.

A chromosome behavior different from that which is characteristic of Aegilops × Triticum hybrids was found by the writers in the study of the meiotic phases of the hybrid *A. crassa* × *T. dicoccoides*. The chromosomes in this hybrid were distributed during the reduction division in a manner similar to that observed in Triticum × Triticum hybrids and unlike that of the other Aegilops × Triticum hybrids studied. This exceptional behavior suggests a closer relationship between *T. dicoccoides* and *A. crassa* than between other Triticum species and *A. crassa*. The extent of the pairing in hybrids between these two genera is similar to that observed in hybrids between einkorn and members of the two polyploid Triticum groups, but the lower amount of pairing suggests a more distant relationship between these genera than that between the Triticum groups described above. The more recent studies of chromosome pairing in hybrids between Aegilops and Triticum give little support to that portion of the hypothesis proposed by Gaines and Aase (*11*) and illustrated in Table 8 to explain the relationship of Aegilops to Triticum.

TABLE 8.—*Chromosome sets contained in Triticum and Aegilops*

Group or species	Gaines and Aase, sets of 7 chromosomes				Present writers, sets of 7 chromosomes				
	A	B	C	D	A	B	C	D	E
Einkorn	×				×				
Emmer	×	×			×	×			
Vulgare	×	×	×		×	×	×		
Aegilops cylindrica			×	×		×		×	
A. ovata					×			×	
A. crassa						×		×	×

The inconsistency of pairing of the A sets of chromosomes in hybrids between einkorn and members of the emmer and *vulgare* groups suggests that the chromosomes in the A sets of the above groups may be similar in some respects but not identical. On the other hand, the consistent pairing of the A and B sets of chromosomes in hybrids between members of the emmer and *vulgare* groups seems to indicate that the chromosomes are more nearly alike.

When *Aegilops ovata* was crossed with einkorn the same inconsistent pairing phenomenon was exhibited as that observed in hybrids between einkorn and members of the emmer and *vulgare* groups. The A set of chromosomes in *A. ovata* does not appear to be identical

with that in einkorn or members of the emmer and *vulgare* groups. Neither does it appear that the B set of chromosomes in the *vulgare* group is identical with that in the emmer group and in *A. cylindrica* and *A. crassa*.

Even more complex relationships exist in the Aegilops groups. From the recent cytological evidence apparently none of the sets in the Aegilops are exactly the same as those in the Triticum groups. The set in *A. cylindrica* now placed under B by the writers is the set previously placed under C by Gaines and Aase (*11*). This change is suggested by the pairing of chromosomes (Table 7) observed in hybrids between *A. cylindrica* and species of the emmer group. By consulting Tables 7 and 8 it may be seen that in hybrids between Aegilops and Triticum there is little if any suggestion of the presence of a C set of chromosomes. It is more apparent, however, from the pairing observed in *A. cylindrica* × *T. turgidum* and *T. polonicum* hybrids that some chromosomes of both A and B sets are present.

From the complete and partial incompatibility of chromosomes exhibited in hybrids resulting from a combination of parents with different numbers of chromosomes, it appears that in any two species only a few chromosomes or probably none at all are identical.

If the *vulgare* wheats were derived by the combination of *Aegilops cylindrica* or *A. ovata* with species of the emmer group, as has been suggested as probable by Percival (*30*), such a condition as that described above makes it difficult to reconcile the cytological results with Percival's view.

The cytological studies of the writers with F_3 hybrid material of the cross *Aegilops ovata* × *Triticum dicoccum* have given evidence of a lack of a true doubling of the chromosome number. If a true doubling takes place like that observed by Tschermak and Bleier (*47*) in their F_5 F_6 Aegilops × Triticum hybrids, the individual plants should produce gametes with 28 chromosomes. None of the F_3 hybrid material of the cross *A. ovata* × *T. dicoccum* examined by the writers has exhibited the doubling phenomenon observed by Bleier, but on the other hand it produced individuals the reduced chromosome number of which was 25 to 27.

A casual inspection of the morphological characters of plants of the F_1, F_2, and F_3 gives the impression that the F_1 type is perpetuated throughout the F_2 and F_3 generations and that no segregation takes place. Limited detailed observations on several plant characters, however, indicate that measurable significant variations may occur among individuals within the F_2 and F_3 generations. From the cytological study of several individuals of the F_3 herein described, a more marked variation in the chromosome behavior is exhibited than that expressed by the morphological character of the same plants. From this it appears that the absent chromosomes do not contain any factors that contribute toward the expression of the major morphological characters exhibited by these individuals. From the meager cytological observations made by the writers with F_3 plants containing from 25 to 27 chromosomes it appears that individuals having a haploid chromosome complement of 1 to 3 less than 28 may exhibit little variation in morphological characters.

That elimination of certain forms with different morphological characters and chromosome numbers takes place and may be expressed

in several ways is suggested by the following observations on self-pollinated F_1, F_2, and F_3 plants of the hybrid *Aegilops ovata* × *Triticum dicoccum:*

(1) Completely sterile, partly sterile, and practically fertile plants have been produced.
(2) No plants have been examined with a chromosome number of less than 25.
(3) A large percentage of apparently normal seeds fails to germinate.
(4) Some plants are stunted or succumb before reaching maturity.
(5) Microscopical examination of the contents of the anthers of various plants shows some plants with almost perfectly developed pollen, whereas others contain imperfectly developed pollen.

SUMMARY

The haploid chromosome number in species of Secale, Triticum, and Aegilops is seven or a multiple of this basic number.

The chromosome behavior during meiosis of the developing pollen mother cell in species of these three genera is regular, producing normal tetrads with each cell receiving the same number of chromosomes.

Very few chromosome abnormalities were observed in hybrids of *Secale cereale* × *S. montanum.*

Pollen mother cells of triploid and tetraploid wheat hybrids show the following abnormalities in chromosome behavior:

Univalent and a few bivalent chromosomes are present in variable numbers in the early heterotypic phases.
Many of the univalents split longitudinally in the heterotypic division.
Only two nuclei and two cells are produced by the heterotypic division.
The homotypic division is characterized by a random distribution and frequent extrusion of the halves of univalent chromosomes that split in the previous division.
Extruded chromosomes frequently form micronuclei.
Tetrads are 4-celled, but many of the cells are polynucleated.

The chromsome behavior in Triticum × Secale and Aegilops × Triticum hybrids shows the following abnormalities:

Univalent and a few bivalent chromosomes are present in varying numbers in the early heterotypic phases.
Occasionally univalents split longitudinally in the heterotypic division.
Frequently univalents collect in the center of the cell without any indication of a mitotic division. This centrally located chromatin mass, however, is often divided in an amitotic manner by the forming cell wall.
The number and size of the cells produced by the heterotypic division is variable.
The second reduction division is more regular than the first. Whenever univalents are present in the chromosome complement they are distributed at random to the poles or are extruded into the cytoplasm.
Tetrads frequently have more than four cells, due to the irregular heterotypic division. Some of the cells of the tetrad may be polynucleated.

The hybrid *Aegilops crassa* × *Triticum dicoccoides* has a chromosome behavior more like that described for Triticum × Triticum hybrids than that of Aegilops × Triticum hybrids.

This exceptional behavior in an Aegilops × Triticum hybrid suggests that *T. dicoccoides* is more closely related than other Triticum species to the genus Aegilops.

F_3 plants of the cross *Aegilops ovata* × *Triticum dicoccum* indicate a tendency toward doubling of chromosome numbers. The few plants examined had from 25 to 27 haploid chromosomes.

The variable chromosome pairing found in hybrids of *Triticum monococcum* and members of the emmer and *vulgare* groups suggests a distant relationship between this species and the polyploid Triticum groups. A more distant relationship than that mentioned above

appears to exist between species of the genus Aegilops and the genus Triticum, as is indicated by the chromosome behavior in hybrids between these genera.

The chromosome behavior in Triticum × Secale hybrids suggests an even more distant relationship between these two genera than between the genera Triticum and Aegilops.

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INHERITANCE OF FUSARIUM RESISTANCE IN CABBAGE[1]

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INTRODUCTION

The diseases of plants caused by vascular Fusaria in many cases have been successfully controlled by the discovery or development, through selection and breeding, of host varieties resistant to attack. Resistant strains of cotton, flax, tomato, cabbage, and other plants are now used in areas where the respective crops are hazarded by this type of malady. In the main these forms have been developed from standard varieties, and in many cases highly resistant strains have been readily secured through mass selection. Comparatively little study has been made of the genetic behavior of Fusarium resistance in the plants mentioned. The investigations of Tisdale [2] in the case of flax wilt indicated that resistance was controlled by multiple factors, but his studies were not extensive enough to warrant final conclusions.

In connection with selection for resistance within several varieties of cabbage which has been under way for a number of years, some attention has been given to this question. This paper is a report of such results as have a bearing upon the nature of inheritance of resistance to yellows caused by *Fusarium conglutinans* Wr.

DEFINITION OF THE PROBLEM

As already noted,[3] highly resistant strains of cabbage have been readily secured by selecting individuals that survive on badly infested soils. A few of such individuals grown in isolation but allowed to cross with one another ordinarily produced progenies showing increased resistance, and by repetition of this process once or twice, a highly resistant strain was usually secured. Complete resistance was not obtained under field conditions, although from the practical standpoint it was easy to maintain a high degree of resistance by selecting seed plants each year from the crop grown on infested soil where the small percentage of susceptible plants was eliminated.

In order to make a critical study of the inheritance of resistance it became necessary to procure, if possible, some lines homozygous for

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[2] TISDALE, W. H. FLAXWILT: A STUDY OF THE NATURE AND INHERITANCE OF WILT RESISTANCE. Jour. Agr. Research 11: 573–606, illus. 1917.

[3] JONES, L. R., and GILMAN, J. C. THE CONTROL OF CABBAGE YELLOWS THROUGH DISEASE RESISTANCE. Wis. Agr. Expt. Sta. Research Bul. 38, 70 p., illus. 1915.

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WALKER, J. C., MONTEITH, J., JR., and WELLMAN, F. L. DEVELOPMENT OF THREE MIDSEASON VARIETIES OF CABBAGE RESISTANT TO YELLOWS (FUSARIUM CONGLUTINANS WOLL.). Jour. Agr. Research 35: 785–809, illus. 1927.

resistance and others homozygous for susceptibility. The first step was to isolate selfed lines from mass-selected resistant lines, on the one hand, and from susceptible commercial varieties on the other. As has already been reported,[4] it was thus possible to secure certain progenies which were completely resistant in the field and others of which the individuals were practically all susceptible. These were then used as parent stocks in crosses between resistant and susceptible lines. The behavior of these crosses and of subsequent generations derived from them, when exposed to the yellows organism, was then studied to determine, if possible, the law which governs the inheritance of resistance in cabbage.

Before discussing the details of experimentation it is important to consider briefly the symptoms of yellows.

SYMPTOMS OF YELLOWS

Yellows is most readily recognized by the characteristic yellowing of the foliage (pl. 1), which appears first in the parenchyma tissue between the veins. It may involve the whole leaf simultaneously, or it may be confined to the portion at one side of the midrib. In the latter case the reduced growth of the diseased portion commonly results in the unilateral development of the organ. Concomitant with or soon following the loss of normal green color, browning of the vascular elements in the diseased tissue can be seen upon cross sectioning. (Pl. 1, B.) Discolored xylem is usually associated with yellowed leaf parenchyma. The diseased parenchyma eventually dies, turns brownish, and becomes dry and brittle. The development of the abscission layer between petiole and stem is hastened, and premature defoliation results. By the time foliar symptoms appear, browning of the vascular system can be traced down the main stem into the taproot and sometimes into the lateral roots. Although the parasite enters through the young roots, there is little or no pathological effect visible upon the root system as a whole. Infection commonly occurs through rootlets on only one side of the underground part of the plant, and in progressing up the stem the fungus may be confined to a few bundles. Thus the unilateral development of the disease in the plant as a whole is common, and in such cases the individual leaves are often unilaterally affected also.

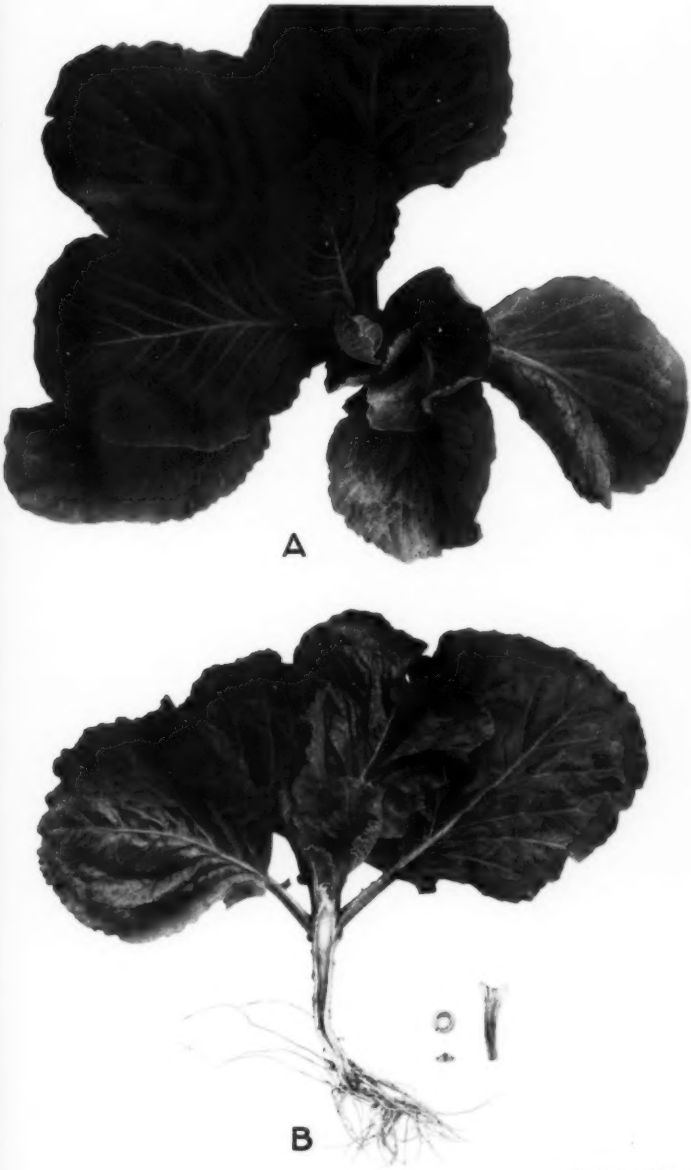
The disease, which is favored by rather high soil temperatures, may develop rather suddenly after the plants are exposed, or very gradually, depending upon the weather. Infected plants may be killed within a few weeks; but if unilateral infection occurs they may survive for a longer period. Gradations of severity are to be noted

[4] WALKER, J. C. STUDIES UPON THE INHERITANCE OF FUSARIUM RESISTANCE IN CABBAGE. (Abstract) Phytopathology 16: 87. 1926.

EXPLANATORY LEGEND FOR PLATE 1

A.—This plant was set in an infested field in southeastern Wisconsin during the last week of June. The photograph was taken about three weeks later. Note characteristic yellowing starting with the lower leaves. Unilateral development of infected leaves is common. The diseased parenchyma tissue later turns brownish in color and becomes dry and brittle. Affected leaves drop off prematurely and plants are commonly killed within a few weeks after the disease appears. Wilting of diseased plants seldom occurs, even in the middle of bright days

B.—A cabbage plant grown on infested soil along with A. The root system shows little or no injury although infection has taken place at the root tips. Browning of the fibrovascular bundles (chiefly in the tracheae of the xylem) is characteristic of the disease and follows closely upon the yellowing of the leaf parenchyma. The fungus is confined to the bundles until the plant has died when it may traverse the dead tissues and fruit upon the surface



EASTERN OFFSET INC. BALTIMORE

CABBAGE YELLOWS

(For explanatory legend, see opposite page)

even up to cases where the symptoms appear on a single lower leaf which eventually drops off, while the plant heads normally without further development of the disease. The number of mild cases is of course greater during cool summers and when late infection is followed by only a short period of favorable temperature. Early, rapidly maturing varieties, well advanced before the soil temperature rises to a point favorable to the disease, usually exhibit fewer severe cases than those planted during the middle of the warm period. The long-season varieties, which even though planted early must normally grow throughout the period of warmest weather before they mature, are likely to show a higher percentage of severely infected individuals than the short-season varieties do. Aside from the diversity in severity of the disease due to purely environmental factors, hereditary differences between individuals of a variety or strain should not be overlooked. These will be given further consideration later in this paper.

Sudden loss of turgidity or wilting is not often the case, as in many herbaceous plants attacked by Fusaria through the xylem. In the greenhouse, when conditions are very favorable for the disease, it sometimes occurs, especially with young plants. In very young seedlings exposed to the disease, particularly in sterilized, artificially inoculated soil, the fungus sometimes invades the cortex at the base of the young hypocotyl, in which secondary thickening has scarcely started. This leads to a typical damping-off symptom involving sudden wilting and death. In the main, these last-described appearances involving loss of turgidity may be regarded as atypical and as usually occurring only under artificial environment.

METHODS OF EXPERIMENTATION

A discussion of the methods of selecting and handling plants for seed propagation has been given in a previous paper [5] and need not be repeated in detail. In the main, plants were grown to seed in the greenhouse during the winter months, and in practically all cases where controlled pollination was practiced they were so handled. In some instances plants of the same genetic constitution, so far as the factors for resistance were concerned, were grown out of doors in an isolated place and allowed to be intercrossed by insects.

These studies were carried on in conjunction with a broader program which included the selection of resistant strains from a number of commercial varieties. Because of the limited greenhouse space it was necessary to handle a large number of seed plants in rather crowded quarters, and manipulation was carried out by a number of different workers. Branches on which flowers were being selfed or crossed were covered with paper bags. Even with reasonable care, however, a slight amount of contamination by foreign pollen was experienced. Where such pollen came from plants of distinctly different type usually the error could be readily detected in the subsequent trials, but this was not always possible. Instances will be cited, therefore, during the course of the discussion of the results where errors through pollen contamination or even through mechanical mixture of seeds of plants are suspected.

[5] WALKER, J. C., MONTEITH, J., Jr., and WELLMAN, F. L. Op. cit.

Tests for resistance or susceptibility discussed in this paper were usually carried out in the field upon thoroughly infested soil. Certain studies carried out subsequently under controlled conditions are discussed in another paper.[6] The main testing ground was one used previously over a period of years during the selection of resistant varieties. It is located in the cabbage-growing area of southeastern Wisconsin (Kenosha County) and was selected in the beginning because of the thorough infestation of the soil with the yellows organism. Repeated cropping with cabbage has made it even more uniformly infested.

Earlier experience had shown that the most general infection under these local conditions occurred usually during the early part of July, especially if plants had been set a short time previously. The soil temperature, as a rule, is very favorable for disease during July and early August. After the middle of August the mean temperature gradually decreases. The seed was sown, therefore, in noninfested soil from about May 15 to June 1, and the plants for the most part were set in the trial plot during the first week of July. In 1927 the seed-bed soil was found to be somewhat infested, inasmuch as a few plants showed slight symptoms at transplanting. Although such plants were not eliminated at transplanting and were set out as they came, no appreciable difference in the results was noted.

After the appearance of the disease the trial plot was examined at frequent intervals and each plant showing symptoms was marked with a bamboo stake which was left until the end of the season. Each diseased plant was thus permanently labeled and could be recorded at any time later, even though it might in the meantime die and disappear or, on the other hand, recover from any external symptom. Since there were often differences in the severity of attack between individuals in a given progeny, and the significance of these differences was not completely understood, the diseased plants were classified at the end of the season into three categories: (1) Those dead or severely diseased; (2) those slightly diseased and continuing to show symptoms at the end of the season; and (3) those which at some time showed slight symptoms but which recovered from any external appearance before the end of the season.

As is usually the case in genetic studies of disease resistance, some difficulty was encountered in the differentiation of plants into two discontinuous classes; that is, healthy (resistant), and diseased (susceptible). It will be seen in the discussion, however, that the percentage of plants occupying the border line between the two classes was comparatively small. A large majority of the plants regarded as susceptible were either killed or severely diseased. A small percentage, averaging usually less than 10 per cent of the diseased plants, fell into the slight class. The plants that showed slight symptoms and recovered were relatively few and comprised on an average less than 3 per cent of the diseased class. There is some question whether the members of this last group should be classed as resistant or susceptible. An occasional plant in the resistant lines showed slight symptoms and recovered. On the other hand, three recovered plants from susceptible or segregating progenies yielded progenies which

[6] WALKER, J. C., and SMITH, R. FACTORS INFLUENCING EXPRESSION OF FUSARIUM RESISTANCE IN CABBAGE. [Unpublished manuscript.]

reacted as did those from susceptible plants. It is possible that if the constitution of all the recovered plants had been studied by progeny tests some might have fallen into each class. Since this procedure was not feasible and the numbers were too small to affect the significance of the ratios, such plants were all placed in the susceptible class.

Further evidence of a well-defined distinction between the susceptible and resistant classes was gained through study of the two types when grown on infested soil under controlled conditions at a constant soil temperature of 24° C. As has been shown in detail in another paper,[7] and by brief discussion herein, the plants of susceptible progenies succumbed promptly and completely at this temperature, whereas the resistant progenies withstood the disease perfectly. Furthermore, when segregating progenies were tested in this manner, the percentage of susceptible plants was practically identical with that in the field tests except that the disease appeared and progressed much more uniformly in the susceptible plants under controlled environmental conditions.

RESISTANT LINES FROM ALL HEAD EARLY AND GLORY OF ENKHUISEN VARIETIES

The first resistant line secured originated with a resistant plant selected in 1921 from an All Head Early progeny segregating into resistant and susceptible plants. This plant, 5–11, was grown to seed out of doors in complete isolation in 1922. The progeny, tested in 1923, showed 507 healthy and no diseased individuals. (Table 1.) Several individuals (5–21, 5–22, 5–24, 5–25, 5–27, 5–28, and 5–29) were selected from this progeny for seed production during the following winter. Certain of these plants were used as parents for resistant-susceptible crosses, while some flowers were self-pollinated and some sib crosses were made. The progenies from self-pollination and sib crosses were tested during 1924 and 1925. The results in Table 1 show that out of 260 second-generation plants only 3 were diseased. Of these, 1 died, 1 was slightly affected, and 1 recovered. Selected plants from the second generation were carried on to seed; selfs and sib crosses were made. With the exception of one selfed progeny (5–39s) [8] and one plant which showed slight symptoms and recovered, all again showed freedom from disease. Plant 5–39 was evidently the result of a contamination. Its selfed progeny contained individuals entirely different in color of foliage and shape of head from the plants in the other progenies. It was, therefore, excluded from the totals.

This line was, with the exceptions noted, completely resistant to yellows and continued to remain so throughout three generations. Since the time of this selection numerous other individuals selected from All Head Early have yielded perfectly resistant progenies when selfed, and where they were continued they were repeatedly resistant in succeeding generations. Resistance remained stable in spite of

[7] WALKER, J. C., and SMITH, R. Op. cit.

[8] Throughout this paper the suffix "s" indicates that the progeny is the result of the self-pollination of a given plant. When a progeny is the result of a cross between two plants, e. g., 5–21×25 (Table 1), the number of the pistillate parent is given first. In some cases the blossom of the pistillate parent was not emasculated, but a brush was worked back and forth between blossoms of paired plants; and such progenies are therefore a mixture of plants resulting from selfing of the pistillate parent and from crossing with another staminate parent. Such a progeny is designated by the suffix B; e. g., H5I–1×2B. (Table 7.)

the fact that in some cases there was a decided reduction in size and vigor of plants due to repeated selfing. There appears to be no correlation of resistance with pronounced vigor in these cases, as pointed out earlier with Wisconsin Hollander.[9]

TABLE 1.—*Occurrence of yellows in progenies of the resistant line selected from the All Head Early variety of cabbage*

Generation	Progeny No.	Number of plants		Generation	Progeny No.	Number of plants	
		Diseased	Healthy			Diseased	Healthy
First	5-11s	0	507	Third	5-32s	0	16
Second	5-21s	[a] 2	92		5-32×34	0	43
	5-21×25	0	53		5-32×44	0	77
	5-22s	0	9		5-33s	0	32
	5-24s	0	7		5-34s	[c] 1	45
	5-25s	0	5		5-34×32	0	48
	5-27s	0	31		5-34×39	0	57
	5-28s	0	13		5-34×44	0	24
	5-28×27	0	38		5-39s [d]	14	45
	5-29s	[b] 1	9		5-44s	0	29
Total		3	257		5-44×45	0	11
					5-45s	0	31
				Total		1	413
				Grand total		4	1,177

[a] 1 slight; 1 recovered.
[b] Severe.
[c] Recovered.
[d] Probably a result of contamination; results not included in totals.

The second resistant line used in making initial crosses was selected from Glory of Enkhuizen. Two plants from a partially resistant line were selfed. One of them yielded a segregating progeny and the other a completely resistant progeny. The homozygous plant, 35–12, was used as a resistant parent. Selections from the selfed progeny of this plant yielded completely resistant progenies, except one which, since it segregated into 25 per cent susceptible and 75 per cent resistant, was interpreted as a chance contamination during the self-pollination of 35–12.

SELECTION OF SUSCEPTIBLE PLANTS

As has been observed above, a large percentage of most commercial varieties of cabbage succumb to yellows when planted upon infested soil. A small number that survive yield, upon self-pollination or crossing within the group, progenies much higher in percentage of resistant individuals than the original variety from which they were selected. In order to secure susceptible individuals to use in crosses with resistant parents, plants of standard commercial varieties growing upon noninfested soil were selected. These were taken from three standard varieties, All Head Early, Glory of Enkhuizen, and Copenhagen Market. The results of progeny tests from self-pollination and crossing between certain individuals are given in Tables 2 to 4.

[9] JONES, L. R., and GILMAN, J. C. Op. cit.

TABLE 2.—*Tests on yellows-infested soil of progenies secured from the All Head Early (commercial cabbage variety) seed plants grown the previous year on noninfested soil*

[H-1, H-2, H-3, H-6, H-11, and H-12 were used as susceptible parents in crosses with resistant parents, Tables 6 to 11]

Source of progeny (self or cross)	Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants diseased	
			Dead or severe	Slight	Recovered	Diseased	Healthy
Selfed progenies	H-1s	1924	44	4	1	49	0
	H-2s	1925	17	2	0	19	6
	H-3s	1925	14	1	6	21	3
	H-4s	1924	0	0	2	2	4
	H-5s	1925	0	2	3	5	16
	H-6s	1924	8	0	0	8	2
	H-7s	1924	11	3	1	15	9
	H-11s	1924	11	1	5	17	4
	H-12s	1924	41	3	0	44	10
Crosses between plants yielding very susceptible selfed progenies.	H-2×1	1925	25	0	0	25	0
		1925	15	3	0	18	0
	H-3×2	1925	25	0	0	25	0
	H-6×1	1924	34	5	11	50	2
		1925	24	1	0	25	0
	H-11×12	1924	67	13	12	92	5
	H-12×11	1924	38	6	2	46	4
Crosses between plants yielding in one case susceptible and in the other segregating selfed progenies.	H-1×4	1924	21	3	9	33	13
		1925	10	0	1	11	15
	H-4×1	1924	26	1	1	28	47
	H-2×4	1925	5	2	1	8	17
	H-3×4	1925	8	2	2	12	13
	H-4×6	1924	36	4	15	55	24
		1925	9	3	0	12	12
	H-6×4	1924	8	5	6	19	21
		1925	13	3	0	16	9
	H-7×11	1924	17	9	3	29	61
		1925	6	1	2	9	16
		1926	21	1	0	22	34
	H-7×12	1924	23	2	1	26	19
		1925	12	0	0	12	12
		1926	20	5	2	27	29

TABLE 3.—*Tests on yellows-infested soil of progenies secured from Glory of Enkhuizen (commercial cabbage variety) seed plants grown the previous year on noninfested soil*

[G-7 and G-15 were used as susceptible parents in crosses with resistant parents, Tables 6 to 11]

Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants	
		Dead or severe	Slight	Recovered	Diseased	Healthy
G-2s	1925	18	5	2	25	0
G-5s	1925	8	0	1	9	1
G-7s	1924	5	1	0	6	0
G-15s	1924	7	0	0	7	0
G-4×12	1925	5	1	2	8	0
G-5×2	1925	23	1	11	35	1
G-7×5	1925	26	2	3	31	0
G-7×13	1924	95	8	10	113	1
G-13×12	1924	30	7	7	44	2
G-14×10	1925	8	1	0	9	0
G-14×12	1925	11	1	1	13	0
G-15×5	1924	28	0	0	28	1
	1925	18	0	0	18	1

TABLE 4.—*Tests on yellows-infested soil of progenies secured from Copenhagen Market (commercial cabbage variety) seed plants grown the previous year on noninfested soil*

Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants	
		Dead or severe	Slight	Recovered	Diseased	Healthy
C–4s	1926	0	1	0	1	11
	1927	14	0	0	14	18
C–4×9	1926	16	1	0	17	17
C–6s	1926	22	2	0	24	4
	1927	14	0	0	14	6
C–7s	1926	27	5	0	32	0
	1927	39	0	0	39	0
C–7×6	1926	32	6	3	41	2
	1927	18	1	2	21	1
C–9s	1926	3	0	0	3	0
C–12s	1926	5	1	0	6	11
	1927	7	0	5	12	4
C–12×H–24	1926	6	2	1	9	0
C–13s	1926	2	0	0	2	4
	1927	4	0	0	4	29
C–13×H–27	1926	0	2	2	4	9
	1927	4	0	6	10	15
C–14s	1926	3	16	7	26	0
C–15s	1927	21	0	0	21	6
C–17s	1927	2	0	13	15	1

In the All Head Early plants an examination of the data (Table 2) shows that plants H–1,[10] H–2, H–3, H–6, H–11, and H–12 when selfed, or crossed with one another, yielded progenies quite susceptible to yellows. Plants H–4, H–5, and H–7 yielded selfed progenies with considerable percentages of resistant plants, and in the progenies from crosses with others in the first group resistant plants were about as numerous as susceptible ones. In the Glory of Enkhuizen group (Table 3) all plants yielded very susceptible progenies whether selfed or crossed with others in the group. In the Copenhagen Market group (Table 4), C–4 and C–13 yielded selfed and hybrid progenies with a high percentage of resistant plants, while the others in the group, except C–12, produced very susceptible families. C–12 might be classed with C–4 and C–13 were it not for the fact that when crossed with a susceptible individual of the All Head Early variety (H–24), a completely susceptible F_1 progeny resulted. As plant C–12 stood close to plant C–13 in the greenhouse it is possible that some pollen contamination occurred while the bags were removed for selfing.

It may be well to consider the behavior of these progenies from susceptible varieties in the light of data which were subsequently secured and presented in this paper and which seem to show that resistance in the cases studied is governed by a single dominant factor. It is suggested that in the picking of plants at random from a commercial variety growing upon yellows-free soil one may expect occasionally to secure a plant which when selfed yields a progeny of much higher resistance than the average of the lot from which it was chosen. On the single-factor basis such an individual may be heterozygous or homozygous for resistance. None of those encoun-

[10] Throughout this paper H denotes progeny developed from the All Head Early variety, C that from Copenhagen Market, G that from Glory of Enkhuizen, and WC that from wild cabbage.

tered in the selections just described were homozygous for resistance. Out of the 31 plants studied, coming from 3 varieties, 5 individuals (H–4, H–5, H–7, C–4, and C–13) seem to have been heterozygous for resistance. This is indicated by the behavior of their selfed progenies and shown more conclusively in the large populations of the hybrids resulting from the crossing of these individuals with others in the series which seemed to be homozygous for susceptibility.

In the case of the remaining plants of the series (excepting C–12) a large share of those in selfed progenies and of the hybrids from crosses within the group were diseased. It is to be noted, however, that all plants were not equally affected. A majority were severely diseased, while a smaller number were slightly affected and a few recovered. A completely satisfactory explanation for this variation can not be given. Under the conditions of the trials it may reasonably be expected, however, that unevenness of distribution of the pathogene in the soil may account for it in part. Some light on the question might be obtained by growing such individuals to seed and testing the progenies. However, little success has been had in carrying mildly affected plants to maturity of seed. Three plants in the recovered class have so far been grown to seed and their progenies tested. All of these yielded progenies which reacted similarly to those from plants known to be susceptible. There is thus no evidence yet at hand to indicate that the plants in this class are necessarily heterozygous or homozygous for resistance, although a study of a larger number might show some to belong to one or another of these two classes.

Two possible explanations may be offered to account for the fact that some plants remained healthy in these very susceptible progenies. Some of them may have accidentally escaped infection, while others may have undergone chance contamination with foreign pollen from heterozygous or homozygous resistant plants. Two surviving healthy plants from H–12×11 were grown to seed. The selfed progenies of each indicated that both of these survivors were heterozygous. One yielded 13 healthy to 7 diseased plants while the other yielded 12 resistant to 4 diseased. A portion of the C–12s progeny was grown on yellows-free soil, and 5 individuals were carried on to seed. Of these, 3 proved to be homozygous for susceptibility and 2 were clearly heterozygous. It is quite likely that in both these progenies, H–12×11 and C–12s, there had been slight contamination by foreign pollen.

Further light on the inheritance of susceptibility was secured by the continuation of certain of the lines. Two plants from progeny H–1s grown on yellows-free soil were grown to seed, and the selfed progenies were tested on infested soil. One of these tested in 1925 and 1926 yielded 99 diseased and 14 healthy plants; the other tested during the same year yielded 72 diseased and 2 healthy. A number of crosses were made in which the staminate and pistillate parents came from different varieties. Some of these are already recorded in Table 4. Three others, with their consequent behavior on infested soil, are given in Table 5. A certain number of the F_1 plants from H–12×G–15 and H–16×C–9 were grown on yellows-free soil and carried through to the F_2 and F_3 generations. In general the percentage of healthy plants that survived when the F_2 and F_3 progenies were tested was low. (Table 5.) In a few cases in the HC series (e. g.,

HC–11×14B) the number of healthy plants was unusually high, but when this progeny was tested the following season no plants survived, indicating that in the first trial the healthy plants merely escaped infection.

TABLE 5.—*Occurrence of yellows in hybrid progenies from crosses between susceptible plants of Glory of Enkhuizen (G), All Head Early (H), and Copenhagen Market (C) varieties of cabbage, with F_2 and F_3 selections made from some of the crosses, when grown upon yellows-infested soil*

Cross	F_1 plants		F_3 plants				F_3 plants		
	Number diseased	Number healthy	Progeny No.	Year tested	Number diseased	Number healthy	Progeny No.	Number diseased	Number healthy
G-7×H-11	64	2					HG-101×102B	22	2
			HG-1s	1927	11	0	HG-102×101B	11	2
H-12×G-15	80	0	HG-2s	1927	27	3	HG-103×105B	23	1
			HG-3s	1927	19	1			
			HG-4s	1927	23	2			
			HG-5s	1927	24	1			
			HG-2×HC-1	1927	24	0			
				1928	61	0			
			HC-2s	1927	42	1			
			HC-3×19B	1927	22	2			
			HC-4s	1927	18	4			
				1928	21	1			
			HC-5s	1927	25	0			
			HC-5×13B	1927	26	0			
			HC-6s	1927	28	0			
			HC-7s	1927	13	2			
			HC-7×18B	1927	38	2			
				1928	50	0			
H-16×C-9	159	3	HC-10×6B	1927	24	1			
				1928	25	0			
			HC-11s	1927	17	8			
			HC-11×14B	1927	14	11			
				1928	15	0			
			HC-12s	1927	16	9			
				1928	20	5			
			HC-13×5B	1927	25	0			
			HC-14s	1927	21	4			
				1928	19	0			
			HC-14×11B	1927	22	3			
				1928	21	0			
			HC-15s	1927	44	4			
				1928	21	0			
			HC-16s	1927	15	1			
				1928	26	1			

Later in this investigation greenhouse tests were made with a number of these progenies, the purpose being to subject the plants to conditions which would more certainly produce uniform infection. Both naturally infested soil from the test plot and soil that had been sterilized and inoculated with a pure culture of the causal organism were used. After the plants had recovered from transplanting the containers were placed in soil temperature tanks at 23°–24° C., where, as previous work had shown, homozygous resistant plants and heterozygous plants remain healthy, while homozygous susceptible plants succumb promptly. When progenies HC–4s, HC–7×18B, HC–11×14B, HC–14s, HC–14×11B, HC–15s, HC–16s, HG–101×102B, and HG–103×105B were submitted to this test all plants become diseased within one week after the cans were placed in the tanks. This would seem to indicate that a great many of the healthy plants that survived in the field tests had escaped infection. Whether

the moderate development of disease in certain of the susceptible plants was due to chance escape of part of the root system from infection, to variations in soil environment, or to hereditary modifying factors remains for the present unsettled. It is probable that each of the three is a potential cause of such variation.

To summarize, it may be stated that most plants selected from the three commercial varieties gave progenies which were very susceptible to the disease. A small number yielded progenies that were much more resistant to yellows than the rest, and these were probably heterozygous plants of the same order as many of those that survive when commercial varieties are planted upon infested soil. The others are regarded for the present as showing evidence of being homozygous for susceptibility.

INHERITANCE OF RESISTANCE

IN ALL HEAD EARLY AND GLORY OF ENKHUIZEN VARIETIES

F_1 Generation

Crosses of resistant plants, 5–21 to 5–29 (derived from All Head Early) and 35–12 (derived from Glory of Enkhuizen) with the susceptible plants H–1, H–2, H–3, H–4, H–5, H–6, H–7, H–11, H–12, G–5, G–7, and G–15 were made. Since H–4, H–5, and H–7 were later shown to be distinctly heterozygous, crosses into which they entered are dropped from further consideration here. The initial hybrid progenies were first tested in 1924, and many were repeated in the trials of the following seasons up to and including 1927. A summary of the records as to their behavior when planted on infected soil is given in Table 6. The dominance of the resistant character is very consistent throughout. Out of a total of 2,773 plants, 24 showed the disease; of these 5 recovered, 1 was slightly affected, and 18 were severly diseased. In several instances the susceptible plants did not appear consistently when the progeny was tested during several seasons. It is quite possible that they resulted from a mechanical mixture of seed or of plants, or they may have been caused by an occasional accidental self-pollination during the process of crossing. It is to be noted in the latter connection that the diseased plants appeared, in all cases but two, where the susceptible plant was used as the pistillate parent, and in these two exceptions the plants showed only slight symptoms and later entirely recovered from any external signs of yellows. The behavior of the resistant and susceptible lines and the F_1 hybrid in the 1925 trial plot is illustrated in Figure 1. In the center is the susceptible progeny, H–3×2 (Table 2), in which all plants were diseased. At the right is the resistant progeny, 5–28×27 (Table 1), which was entirely healthy. At the left is the hybrid progeny, H–1×5–23 (Table 6), which was equal in resistance to the homozygous resistant progeny, 5–28×27.

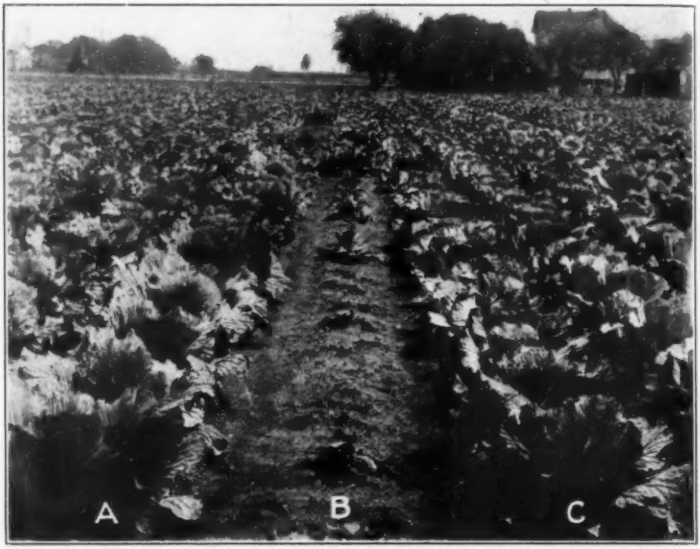


FIGURE 1.—Comparison of homozygous resistant, homozygous susceptible, and F_1 hybrid progenies on the naturally infested field-trial plot in Kenosha County, Wis., in 1925. A, Cross between susceptible and resistant plants (H-1×5-23, Table 6); all plants remained healthy. B, Susceptible progeny (H-3×2, Table 2); all plants were diseased. C, Resistant progeny (5-28×27, Table 1); all plants were healthy

TABLE 6.—*Occurrence of yellows in F_1 hybrid progenies resulting from crosses between homozygous resistant and homozygous susceptible cabbage plants when grown in the field upon infested soil*

Cross	Year tested	Number of plants	
		Diseased	Healthy
H-1×5-23	1924	0	51
	1925	0	26
	1926	0	62
H-1×5-24	1924	[a] 4	59
	1925	[b] 2	67
	1926	0	31
5-24×H-1	1924	0	15
H-1×5-25	1924	0	56
	1925	0	107
	1926	0	47
5-25×H-1	1924	0	52
H-1×5-27	1924	1	77
	1925	0	49
	1926	0	48
5-27×H-1	1924	0	12
H-1×5-28	1924	0	79
	1925	0	29
H-1×5-30	1924	[b] 1	59
	1925	0	26
H-2×5-23	1925	0	51
H-2×5-24	1925	0	18
5-25×H-2	1924	0	30
H-2×5-27	1925	0	35
H-2×5-28	1925	0	20
5-28×H-2	1926	0	25
H-2×5-30	1925	0	31
H-3×5-23	1925	0	24
H-3×5-24	1925	[b] 1	15
H-3×5-27	1925	0	28
H-3×5-28	1925	0	24
H-3×5-30	1925	0	25
H-6×5-23	1924	0	42
	1925	0	25
H-6×5-24	1924	0	43
	1925	0	25
H-6×5-25	1924	0	58
H-6×5-27	1924	0	14
H-11×5-21	1924	0	63
5-21×H-11	1924	[c] 1	66
H-11×5-25	1924	[d] 1	129
5-25×H-11	1924	[c] 1	64
H-11×5-26	1924	0	70
	1925	0	28
H-12×5-21	1924	0	82
	1925	0	73
5-21×H-12	1924	0	42
H-12×5-24	1924	0	45
H-12×5-25	1925	[b] 1	54
5-25×H-12	1924	0	60
H-12×5-26	1924	[b] 1	58
G-5×5-24	1925	0	26
G-5×5-25	1925	0	25
G-5×5-26	1925	[b] 4	22
G-7×35-12	1924	0	50
G-15×5-21	1925	[b] 2	24
G-15×5-24	1924	[c] 1	52
	1925	[d] 1	32
G-15×5-25	1924	0	51
	1925	0	71
G-15×5-26	1924	[e] 2	53
	1925	0	24
Total		24	2,749

[a] 1 dead; 1 severe; 1 slight; 1 recovered.
[b] Dead.
[c] Recovered.
[d] Severe.
[e] 1 dead; 1 recovered.

F_2 Generation

Plants were selected from a number of progenies from the F_1 hybrid tests in 1924 and from others in 1925. Those from the following progenies were grown to seed in the greenhouse: H–1×5–24, 5–24×H–1, H–1×5–25, 5–25×H–1, G–15×5–24, and G–15×5–25. Seed from self-pollination was secured whenever possible. Inasmuch as the number of seeds from selfing is often very low, a larger quantity of seed was insured by making sib crosses between plants of the same parentage. These were secured by merely working a camel's-hair brush over blossoms of paired plants, or in some cases a number of heads from a single cross were carried over winter and planted in an isolated group out of doors the following summer. In cases like the latter the seed from all the plants of the lot was usually mixed. The F_2 progeny tests were made in the trial plot described before, care being taken to select as uniformly infested soil as possible and to plant at times favorable for infection. As was noted in the suceptible lines, all plants that became diseased did not show equally severe infection. Records were therefore kept according to the arbitrary classification of diseased plants explained above. The first F_2 progeny trials were made in 1925 and were continued during 1926, 1927, and 1928.

In Table 7 are given the results with the F_2 progenies from the reciprocal crosses, H–1×5–24 and 5–24×H–1. The large majority of the diseased plants fell into the severe class. Variation in the rate of development of the disease in the susceptible group was therefore no greater, in fact it was less, than in the selfed progeny of the susceptible parent shown in Table 2. It is evident that every progeny segregated as a monohybrid, yielding close to 25 per cent diseased plants. The deviation from the theoretical 3 to 1 ratio was usually negligible, while in practically every case where it was more than three times the probable error repetition in another year gave a reasonably close fit. The progenies from sib crosses reacted essentially the same as those from selfed blossoms. One selfed progeny (H5I–4s) [11] showed a poor fit in each of three years. It should be noted, however, that in 1925 and 1927 the number of susceptible plants was higher, while in 1926 it was lower, than the expected. This irregularity may have been due in part to variation in the infestation of the plot, although this is the only progeny which gave such a poor fit consistently. In the sib cross between this plant and another (H5I–5, segregation in the selfed progeny of which was a reasonably close fit) the deviation was less than twice the probable error.

[11] Throughout this paper the use of I in the number designates an F_1 plant from a resistant-susceptible cross or an F_2 progeny from such a plant; i. e., the F_1 hybrid plants selected for seed propagation are each given a number including the letter I. Thus the F_2 progenies therefrom are designated by the parent plant number with the appropriate suffix to indicate whether the seed was secured by self-pollination or by crossing. In this instance H5I–4 is the number given the F_1 bybrid plant. H5I–4s is the number of the F_2 self-progeny derived from that plant.

TABLE 7.—*Occurrence of yellows in F_2 cabbage progenies from the reciprocal crosses, H–1×5–24 and 5–24×H–1 and H–1×5–25 and 5–25×H–1 (Table 6), when grown in the field upon infested soil*

Original cross No. and kind of cross	Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants		Deviation	Probable error	Dev. / P. E.
			Dead or severe	Slight	Recovered	Diseased	Healthy			
H–1×5–24: Self	H5I–1s	1925	12	0	0	12	37	0.3	2.0	0.2
	H5I–2s	1925	17	2	0	19	40	4.3	2.2	2.0
		1926	34	1	0	35	82	5.8	3.2	1.8
	H5I–3s	1925	7	1	1	9	27	0	1.8	0
		1926	20	0	1	21	73	2.5	2.8	.9
	H5I–4s	1925	24	0	2	26	39	9.8	2.4	4.1
		1926	9	1	0	10	82	13.0	2.8	4.6
		1927	10	1	0	11	15	4.5	1.5	3.0
	H5I–5s	1925	11	0	3	14	43	.3	2.2	.1
		1926	20	3	0	23	57	3.0	2.6	1.2
	H5I–6s	1926	24	3	0	27	91	2.5	3.2	.8
		1927	22	3	0	25	34	10.3	2.2	4.7
Total			210	15	7	232	620	19.0	8.5	2.2
Sib	H5I–1×2B	1926	4	0	0	4	17	1.3	1.3	1.0
	H5I–2×1B	1925	10	1	0	11	31	.5	1.9	.3
	H5I–3×1B	1926	20	0	0	20	65	1.3	2.7	.5
	H5I–4×5B	1926	19	1	1	21	83	5.0	3.0	1.7
	H5I–5×4B	1925	12	0	2	14	40	.5	2.2	.2
Total			65	2	3	70	236	6.5	5.1	1.3
5–24×H–1: Self	5HI–4s	1925	7	0	0	7	40	4.8	2.0	2.4
		1926	33	1	0	34	69	8.3	3.0	2.8
	5HI–6s	1925	18	4	0	22	67	.3	2.8	.1
		1926	23	1	2	26	97	4.8	3.2	1.5
		1927	11	0	0	11	24	2.3	1.7	1.4
Total			92	6	2	100	297	.8	5.8	.1
Sib	5HI–2×5B	1925	7	5	0	12	46	2.5	2.2	1.1
	5HI–6×4B	1925	15	2	0	17	46	1.3	2.3	.6
		1926	24	1	0	25	83	2.0	3.0	.7
Total			46	8	0	54	175	3.3	4.4	.8
Grand total			413	31	12	456	1,328	10.0	12.3	.8
Grand total calculated on 1 : 3 ratio						446	1,338			
H–1×5–25: Self	H5I–12s	1925	4	2	0	6	49	7.8	2.2	3.5
		1926	20	0	3	23	83	3.5	3.0	1.2
		1927	0	2	2	4	22	2.5	1.5	1.7
	H5I–14s	1925	17	3	2	22	45	5.3	2.4	2.2
		1926	16	0	0	16	44	1.0	2.3	.4
Total			57	7	7	71	243	7.5	5.2	1.4
Sib	H5I–12×14B	1925	7	0	0	7	41	5.0	2.0	2.5
		1926	23	1	0	24	76	1.0	2.9	.3
	H5I–14×12B	1925	6	0	0	6	16	.5	1.4	.4
		1926	15	0	1	16	33	3.8	2.0	1.9
Total			51	1	1	53	166	1.8	4.3	.4
5–25×H–1: Self	5HI–12s	1925	16	3	4	23	59	2.5	2.6	1.0
		1926	7	0	0	7	33	3.0	1.9	1.6
	5HI–14s	1925	5	0	0	5	20	1.3	1.5	.9
Total			28	3	4	35	112	1.8	3.5	.5
Sib	5HI–11×14B	1926	10	1	0	11	31	.5	1.9	.3
	5HI–14×11B	1926	15	0	0	15	70	6.3	2.7	2.3
	5HI–12×13B	1925	7	1	0	8	32	2.0	1.9	1.1
		1926	17	4	0	21	63	0	2.7	0
Total			49	6	0	55	196	7.8	4.6	1.7
Grand total			185	17	12	214	717	18.8	8.9	2.1
Grand total calculated on 1 : 3 ratio						233	698			

TABLE 8.—*Occurrence of yellows in F^2 cabbage progenies from the crosses, G–15×5–24, G–15×5–25, and G–7×35–12 (Table 6), when grown in the field upon infested soil*

Original cross No. and kind of cross	Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants		Deviation	Probable error	Dev. / P. E.
			Dead or severe	Slight	Recovered	Diseased	Healthy			
G–15×5–24: Self	G5I–1s	1925	5	0	0	5	34	4.8	1.8	2.7
	G5I–14s	1926	10	0	0	10	37	1.8	2.0	.9
		1927	21	0	0	21	27	9.0	2.0	4.5
	G5I–16s	1926	11	3	0	14	33	2.3	2.0	1.2
	G5I–17s	1926	11	2	0	13	34	1.3	2.0	.7
	G5I–21s	1927	11	0	0	11	46	3.3	2.2	1.5
Total			69	5	0	74	211	2.8	4.9	.6
G–15×5–25: Self	G5I–6s	1925	31	2	2	35	93	3.0	3.3	.9
	G5I–23s	1927	15	0	0	15	35	2.5	2.1	1.2
	G5I–28s	1926	10	0	0	10	39	2.3	2.0	1.2
Total			56	2	2	60	167	3.3	4.4	.8
Sib	G5I–4×5B	1925	7	0	0	7	41	5.0	2.0	2.5
		1926	15	0	0	15	34	2.8	2.0	1.4
	G5I–5×4B	1925	27	2	1	30	66	6.0	2.9	2.1
		1926	11	0	0	11	36	.8	2.0	.4
Total			60	2	1	63	177	3.0	4.5	.7
G–7×35–12: Self	G35I–7s	1925	5	0	0	5	15	.0	1.3	0
		1926	3	0	0	3	12	.8	1.1	.7
Sib	G35I–10	1926	40	0	0	40	108	3.0	3.6	.8
	G35I–11	1926	27	2	1	30	80	2.5	3.1	.8
	G35I–12	1926	23	7	0	30	77	3.3	3.0	1.1
	G35I–13	1926	34	5	2	41	127	1.0	3.8	.3
		1927	12	3	0	15	39	1.5	2.2	.7
	G35I–14	1926	57	0	0	57	130	10.3	4.0	2.6
		1927	17	1	0	18	31	5.8	2.0	2.9
Total			218	18	3	239	619	24.5	8.6	2.8
Grand total			403	27	6	436	1,174	33.5	11.7	2.9
Grand total calculated on 1:3 ratio.						402	1,208			

The F_2 progeny tests from the reciprocal crosses H–1×5–25 and 5–25×H–1, are summarized in Table 7. The segregation is again consistently that to be expected on the monohybrid basis.

The progenies discussed so far are from crosses where the resistant and susceptible parents were both derived originally from All Head Early. In Table 8 are the results with F_2 progenies from two crosses wherein a Glory of Enkhuizen plant (G–15) was used as the susceptible parent and All Head Early plants (5–24 and 5–25) were used as resistant parents. Results with progenies from a cross between a susceptible and a resistant plant, both derived from Glory of Enkhuizen (G–7×35–12), are also included in this table. In the last case only one plant from a selfed plant was secured, but a number of F_1 plants were carried through winter storage and set out of doors the following spring in an isolated location. Cross-fertilization by bees went on within the group, and the seed from each plant was saved separately. These progenies (G35I–10 to 14) were also tested and the results are included in Table 8. In all the progenies from these three crosses there is close conformity to simple segregation into 3 resistant to 1 susceptible. The comparatively small proportion of

diseased plants falling within the slight and recovered groups is also worthy of notice.

Many more plants were saved from the 1924 tests of F_1 progenies than could be grown to seed the following winter in the greenhouse. The surplus plants were therefore stored during the winter, and the following spring plants from the same cross or closely related crosses were grouped together for seed growing in isolated areas out of doors during the summer of 1925. The seed from the individual plants within each lot was combined. These strains were tested during 1926, 1927, and 1928. The results are assembled in Table 9. Although a wider range of susceptible and resistant parents are included in the crosses represented here, it is evident that the F_2 distributions are in as close agreement with the expectation on the monohybrid basis as those recorded in the two previous tables.

The data concerning the tests of F_2 progenies coming from resistant-susceptible crosses of All Head Early and Glory of Enkhuizen plants and given in detail in Tables 7 to 9 are summarized in Table 10. The total figures for each of four consecutive seasons show very close fits, indicating that the conditions under which the trials were made were perhaps as uniform as could be reasonably expected under field conditions. Variation within the susceptible group as to severity of disease was no greater than that previously described in the susceptible lines.

TABLE 9.—*Occurrence of yellows in F_2 cabbage progenies from several groups of F_1 plants in the field when grown upon infested soil*

[Plants from a single cross or closely related crosses were grown in isolation, natural cross pollination with each group being permitted; the seed from all plants within a single group was combined into one lot]

Original cross	F_2 progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants		Deviation	Probable error	Dev. P. E.
			Dead or severe	Slight	Recovered	Diseased	Healthy			
H-1×5-25	H5IA	1926	25	2	0	27	70	2.8	2.9	1.0
		1927	11	3	1	15	34	2.8	2.0	1.4
		1928	17	4	0	21	75	3.0	2.9	1.0
5-25×H-1	5HIA	1926	28	5	2	35	87	4.5	3.2	1.4
		1927	38	7	0	45	157	5.5	4.2	1.3
H-6×5-23	H5IB	1926	16	0	0	16	32	4.0	2.0	2.0
H-6×5-24		1927	7	0	2	9	38	2.8	2.0	1.4
H-6×5-27										
H-11×5-25	H5IC	1926	8	1	0	9	21	1.5	1.6	.9
		1927	8	0	0	8	13	2.8	1.3	2.2
H-12×5-21	H5ID	1926	62	13	5	80	223	4.3	5.1	.8
H-12×5-24		1927	53	10	1	64	230	9.5	5.0	1.9
H-12×5-25		1928	87	15	0	102	284	5.5	5.7	1.0
H-12×5-26										
G-15×5-25	G5IA	1926	9	0	0	9	13	3.5	1.3	2.7
G-15×5-24	G5IB	1928	6	1	0	7	38	4.3	2.0	2.2
G-15×5-25										
G-7×35-12	G35IA	1928	8	1	0	9	41	3.5	2.1	1.7
G-15×5-24	G5IC	1928	40	0	0	40	97	5.8	3.4	1.7
Total			423	62	11	496	1,453	8.8	12.9	.7
Total calculated on 1:3 ratio.						487	1,462			

TABLE 10.—*Summary of segregation of F_2 cabbage progenies from crosses between resistant and susceptible lines of All Head Early and Glory of Enkhuizen varieties when grown upon infested soil*

Cross	Number of plants with the indicated grades of disease			Total number of plants		Deviation	Probable error	Dev. / P. E.
	Dead or severe	Slight	Recovered	Diseased	Healthy			
H-1×5-24	275	17	10	302	856	12.5	9.9	1.3
5-24×H-1	138	14	2	154	472	2.5	7.3	.3
H-1×5-25	108	8	8	124	409	9.3	6.7	1.4
5-25×H-1	77	9	4	90	308	9.5	5.8	1.6
G-15×5-24	69	5	0	74	211	2.8	4.9	.6
G-15×5-25	116	4	3	123	344	6.3	6.3	1.0
G-7×35-12	218	18	3	239	619	24.5	8.6	2.8
Miscellaneous	423	62	11	496	1,453	8.8	12.9	.7
Total for 1925	287	29	17	333	998	.3	10.6	.03
Total for 1926	783	57	18	858	2,491	20.8	16.9	1.2
Total for 1927	236	30	6	272	745	17.8	9.3	1.9
Total for 1928	118	21	0	139	438	5.3	7.0	.8
Grand total	1,424	137	41	1,602	4,672	33.5	23.1	1.5
Grand total calculated on 1:3 ratio				1,569	4,705			

CROSSES BETWEEN F_1 HYBRIDS AND SUSCEPTIBLE PLANTS

A few back crosses were made between F_1 hybrids and susceptible plants. The occurrence of yellows in these progenies when tested upon infested soil is recorded in Table 11. In practically all cases there was a reasonably close fit to the 1:1 segregation expected. The behavior of the F_1, F_2, and back-crossed progenies in the 1927 trial plot is shown in Figure 2.

TABLE 11.—*Occurrence of yellows in back crosses of F_1 cabbage hybrids (resistant and susceptible) with susceptible individuals*

Progeny No.	Year tested	Number of plants with the indicated grades of disease			Total number of plants		Deviation	Probable error	Dev. / P. E.
		Dead or severe	Slight	Recovered	Diseased	Healthy			
H5I-1×C-1	1925	10	0	0	10	20	5.0	1.9	2.6
H5I-1×H-35	1925	8	0	0	8	8	0	1.4	0
H5I-2×C-1	1925	25	2	0	27	25	1.0	2.4	.4
	1926	20	4	0	24	50	13.0	2.9	4.5
H5I-2×H-35	1926	21	4	1	26	23	1.5	2.4	.6
	1925	24	3	0	27	24	1.5	2.4	.6
H5I-12×C-1	1925	12	4	5	21	33	6.0	2.5	2.4
	1926	30	3	0	33	59	13.0	3.2	4.1
H5I-12×H-35	1926	13	4	0	17	33	8.0	2.4	3.3
	1927	15	6	0	21	20	.5	2.2	.2
H5I-14×H-35	1925	14	0	1	15	25	5.0	2.1	2.4
5HI-5×H-1	1925	13	3	0	16	11	2.5	1.8	1.4
5HI-5×C-1	1925	29	2	1	32	17	7.5	2.4	3.1
	1926	8	2	0	10	14	2.0	1.7	1.2
5HI-11×C-1	1926	23	0	0	23	14	4.5	2.1	2.1
	1925	17	0	0	17	25	4.0	2.2	1.8
5HI-12×C-2	1925	9	3	2	14	19	2.5	1.9	1.3
	1926	11	2	0	13	13	0	1.7	0
C-12×G5I-13	1926	19	0	0	19	16	1.5	2.0	.8
G5I-14×C-12	1926	15	8	2	25	24	.5	2.4	.2
	1927	40	0	0	40	37	1.5	3.0	.5
C-12×G5I-14	1926	20	0	0	20	31	5.5	2.4	2.3
	1927	5	0	0	5	6	.5	1.1	.5
C-12×G5I-28	1926	17	5	0	22	25	1.5	2.3	.7
	1927	16	0	0	16	16	0	1.9	0
Total					501	588	43.5	11.1	3.9
Total calculated on 1:1 ratio					544	544			

F_3 Generation

Heavy losses of seed plants during the winter months of 1925–26 and 1926–27 from causes other than yellows delayed the study of the F_3 generation of these crosses. In the fall of 1927 about 100 F_2 plants were selected at random from plantings upon noninfested soil and removed to the greenhouse. About 30 of these produced seed. As a whole the seed plants were quite vegetative and many of them set little or no seed upon selfing. In order to test the genetic constitution of such plants and to provide a larger population for F_3 trials, back

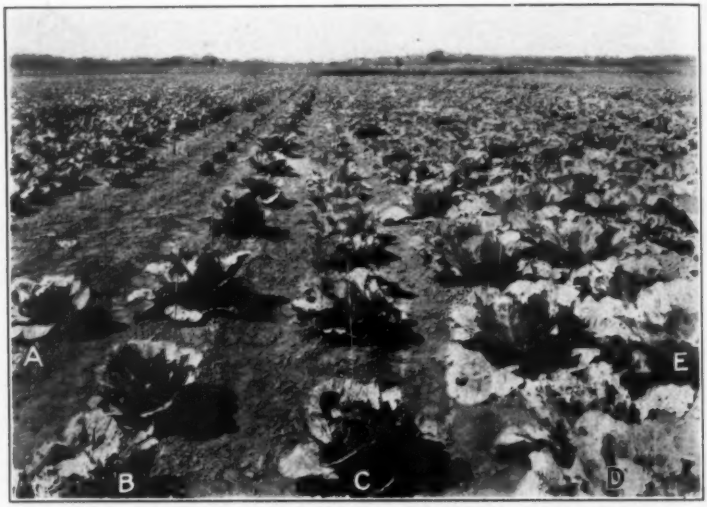


FIGURE 2.—Comparison of susceptible progenies, F_1 hybrids from a susceptible resistant cross, 2 F_2 progenies, and a back cross of an F_1 hybrid plant with a susceptible plant. Trials on naturally infested soil in Kenosha County, Wis., in 1927. A, Susceptible progenies, C–7×6, C–7s, and C–6s (Table 4); all plants except 1 were diseased. B, Back cross of an F_1 hybrid plant, G5I–14, with a susceptible plant, C–12 (Table 11); 40 plants were diseased and 37, healthy. C, F_2 progeny, G5I–21s (Table 8); 11 plants were diseased, 46 were healthy. D, F_2 selfed progeny of the F_1 plant used in the back cross shown in B, G5I–14s (Table 8); 21 were diseased and 27, healthy. This was not a close fit to the expected 3:1 ratio. However, in the 1926 trial on the same field this progeny segregated into 10 diseased and 37 healthy. E, F_1 progeny from a susceptible-resistant cross, H8×5–28; all plants were healthy

crosses were made in many instances, using pollen from plants known to be homozygous for susceptibility.

The seed of these plants did not mature in time to permit field tests to be carried out in the summer of 1928. Since in the meantime studies on the expression of resistance under controlled environment had shown that reliable tests for susceptible and resistant plants could be made in the greenhouse, these F_3 progeny tests were carried out in soil-temperature tanks. Soil used in several previous experiments and known to be thoroughly infested was used. The seedlings were started in noninfested soil and transplanted to the infested soil about three weeks later. After the plants had recovered from transplanting the soil temperature was raised to 23°–24° C. and held there.

The data collected from these trials (Table 12) include the results from selfed progenies and back crosses. In some cases the behavior of

only the back crosses was available to indicate the constitution of a given plant. In some cases also the population was smaller than might have been desired, but where the segregation was significant they were included in the totals. In three cases (5HII–2, G5II–7, and G35II–1) the parent plant was classed as homozygous susceptible, although one or two plants survived out of rather large populations in the selfed progenies. No satisfactory explanation can be offered for these exceptions. It is not believed that they escaped infection, since the soil used was thoroughly inoculated and the environal conditions were ideal.

The four plants in these three progenies were undoubtedly resistant and obviously should not be present if they were true selfs from homozygous susceptible parents. For the present it may only be assumed that these were the result of pollen contamination.

Aside from the exceptions just noted and the back cross of H5II–5, the distribution of homozygous and heterozygous plants is about what might be expected from a monohybrid. It would be desirable, of course, to have a much larger number of F_2 plants tested, and this indeed would have been the case had not the unexpected complications already explained arisen.

TABLE 12.—*Occurrence of yellows in F_3 cabbage progenies and in progenies of F_2 plants crossed with susceptible plants*

[Tests made in the greenhouse upon thoroughly infested soil in soil-temperature-control tanks held at 23°–24° C]

Original cross	F_2 plant No.	Selfed progenies		Back crosses with susceptible plants		Genotype class [a]
		Healthy plants	Diseased plants	Healthy plants	Diseased plants	
		Number	*Number*	*Number*	*Number*	
H-1×5-24	H5II–1			0	4	*rr*
	H5II–2	0	73			*rr*
	H5II–3	47	14			*Rr*
	H5II–4			105	0	*RR*
	H5II–5			14	2	*Rr*
H-1×5-25	H5II–14			2	4	*Rr*
	H5II–17			0	10	*rr*
	H5II–22			6	5	*Rr*
5-24×H-1	5HII–1	11	0			*RR*
	5HII–2	1	98			*rr*
	5HII–3			3	3	*Rr*
	5HII–4	3	0	8	7	*Rr*
	5HII–5			0	8	*rr*
	5HII–6	0	6			*rr*
	5HII–7	27	0	62	0	*RR*
	5HII–8	10	0			*RR*
	5HII–9	55	14	50	55	*Rr*
	5HII–10	61	17			*Rr*
	5HII–11	10	15			*Rr*
	5HII–13			77	0	*RR*
	5HII–21			6	8	*Rr*
G-15×5-24	G5II–1			10	0	*RR*
	G5II–3	0	1	39	42	*Rr*
	G5II–4	27	6			*Rr*
	G5II–7	2	76	0	15	*rr*
	G5II–8			15	0	*RR*
	G5II–9	87	22			*Rr*
	G5II–12			20	22	*Rr*
G-7×35-12	G35II–1	1	23	0	15	*rr*
	G35II–2	20	7	12	7	*Rr*
	G35II–3	7	0	28	30	*Rr*
	G35II–6			13	12	*Rr*

[a] The actual number in each genotype class was *RR* (resistant), 7; *Rr* (heterozygous), 17; *rr* (susceptible), 8; and the expected numbers were 8, 16, and 8, respectively.

Discussion of Crosses Between All Head Early and Glory of Enkhuizen

When the behavior of the parent lines involved in these crosses of the F_1 hybrid and of the F_2 and F_3 families are considered, there seems little doubt that in these cases at least resistance is controlled by a single dominant Mendelian factor. It would be presumptuous to conclude, however, that this is generally the case in cabbage or its allies susceptible to this disease. One should also bear in mind the possibility of variation in the pathogene and the influence of environment upon the expression of resistance by the host. Attention has been called in the course of this report to variation in the response of plants in the susceptible class. How much of this variation is due to environment, and how much is due to heredity? May there enter in modifying factors which though failing to preclude infection and appearance of symptoms do retard progress of the disease? These and other questions are still open and worthy of further study. During the remainder of this paper evidence accumulated in other varieties along the line of that already presented will be discussed. The full discussion of the relation of environment to the expression of resistance is reserved for another paper.[12]

IN JERSEY WAKEFIELD VARIETY

In the fall of 1925 a few surviving healthy plants remained in the infested trial plots from a row of the commercial Jersey Wakefield variety. About 20 of these were planted in the greenhouse and 13 produced seed. Selfed progenies were secured from each, and four sib crosses were made. These were tested on the trial plot during 1926 and 1927. The data are given in Table 13. In all the selfed progenies and in all the sib crosses segregation was reasonably close to the ratio of 3 resistant to 1 susceptible. This indicated that, as already shown in All Head Early and Glory of Enkhuizen varieties, the few individuals that survived on infested soil were heterozygous for resistance. It also indicated that resistance is inherited in this variety in the same manner as in the other two studied.

Table 13.—*Occurrence of yellows in first selections from the Jersey Wakefield variety of cabbage when grown upon infested soil*

Progeny No.	Number of plants		Deviation	Probable error	Dev. / P. E.
	Diseased	Healthy			
20-3s	4	21	2.3	1.5	1.5
20-6s	32	63	8.3	2.9	2.9
20-7s	24	69	.8	2.8	.3
20-8s	10	22	2.0	1.7	1.2
20-9s	20	48	3.0	2.4	1.3
20-10s	49	228	20.3	4.9	4.1
20-12s	33	111	3.0	3.5	.9
20-15s	9	68	10.3	2.6	4.0
20-17s	2	19	3.3	1.3	2.5
20-19s	5	12	.8	1.2	.7
20-21s	69	194	3.3	4.7	.7
20-29s	12	49	3.3	2.3	1.4
20-34s	23	62	1.8	2.7	.7
20-6×3	7	29	2.0	1.8	1.1
20-7×10	58	130	11.0	4.0	2.8
20-11×12	17	33	4.5	2.1	2.1
20-21×15	33	97	.5	3.3	.2
Total	407	1,255	8.5	11.9	.7
Total calculated on 1:3 ratio	415	1,247			

[12] Walker, J. C., and Smith, R. Op. cit.

Additional evidence on this point was secured in subsequent generations. Further selections were made from the surviving plants of the following progenies: 20–7s, 20–10s, 20–15s, 20–21s, 20–7×10, and 20–21×15. Seventeen plants in all were carried through, and the F_2 progenies tested. Where selfed progenies were not secured, the genetic constitution of the plants was tested by the behavior of back crosses with susceptible plants. Since in the parent progenies the susceptible plants, approximating 25 per cent of the total population in number, were eliminated by disease, it would be expected that of the remaining healthy plants approximately one-third would be homozygous for resistance and the remainder heterozygous. The data secured from the field tests of the progenies from selfed plants and those from back-crossed plants are given in Table 14. Six plants were homozygous for resistance, as shown by the behavior on selfing and back crossing to susceptible individuals. Of the remaining 11 plants, those that were tested by selfing segregated close to the 3 : 1 ratio. In the 1927 trials the plants from back crosses were grown on a recent addition to the plot where infestation with the causal organism was not so complete, and this resulted in a somewhat lower percentage of disease than was expected. When plants of which seed was still available were tested in 1928, the segregation was close to a 1 : 1 ratio.

In 1927 one homozygous plant (20–124) was crossed with a susceptible plant of Copenhagen Market (C–15) the selfed progeny of which was 100 per cent susceptible. (Table 4.) This hybrid progeny showed 1 slightly diseased plant out of 38. One of the F_1 plants of this cross (C–15×20–24) was selfed and the F_2 tested in 1928. Of a total of 43 plants, 12 were diseased and 31 healthy (theoretical, 10.7 and 32.3).

TABLE 14.—*Occurrence of yellows in second-generation selections from the Jersey Wakefield variety of cabbage when selfed, and when crossed with susceptible plants; tests made upon infested soil*

Class	Plant No.	Selfed progenies (1927)		Hybrid progenies			
				1927		1928	
		Diseased plants	Healthy plants	Diseased plants	Healthy plants	Diseased plants	Healthy plants
		Number	*Number*	*Number*	*Number*	*Number*	*Number*
Homozygous (*RR*) [a]	20–116	0	30				
	20–117	0	34	0	27		
	20–124	0	20	[b] 1	37		
	20–135	0	7	0	21		
	20–140	0	44	0	27		
	20–154	0	32	0	40		
Total		0	167	1	152		
Heterozygous (*Rr*) [c]	20–115	8	22	17	27	24	17
	20–137	6	20	4	9	33	22
	20–138	6	7				
	20–155	11	21	8	49		
	20–157	17	42	15	34		
	20–158	9	27	9	34		
	20–109			16	34	27	35
	20–131			9	18	20	28
	20–136			12	14	15	13
	20–159			11	7		
	20–139			3	19	18	18
Total		57	139	104	245	137	133

[a] Theoretical number, 5.6. [b] Recovered. [c] Theoretical number, 11.3.

Thus it is clear that in the Jersey Wakefield variety resistance is inherited as a single dominant factor in the same manner as in All Head Early and Glory of Enkhuizen.

IN COPENHAGEN MARKET VARIETY

In 1925 a number of resistant individuals were selected from a planting of Copenhagen Market variety on infested soil. As in the case of Jersey Wakefield, selfs and sib crosses were made during the following winter, and progeny tests were made in 1926 and 1927. The data given in Table 15 show that in all cases but two (21–27s and 21–49s), where the population was small, segregation was close to the ratio of 3 resistant to 1 susceptible. Of the surviving plants in these trials, 8 were grown to seed and their genetic constitution tested by back crossing with susceptible plants. Since the homozygous susceptible plants presumably had been eliminated by disease, approximately one-third of these should have been homozygous resistant and two-thirds heterozygous. This test showed 3 to be homozygous resistant and 5 to be heterozygous. The inheritance of resistance in this variety of cabbage is, therefore, similar to that of other varieties studied.

TABLE 15.—*Occurrence of yellows in first selections of resistant individuals from Copenhagen Market (commercial variety of cabbage) showing 3:1 segregation when grown upon infested soil*

Progeny No.	Number of plants		Deviation	Probable error	Dev. / P. E.
	Diseased	Healthy			
21–27s	10	11	4.8	1.3	3.7
21–28s	50	165	3.8	4.3	.9
21–31s	12	45	2.3	2.2	1.0
21–32s	2	11	1.3	1.1	1.2
21–33s	2	5	.3	.8	.4
21–44s	4	19	1.8	1.4	1.3
21–49s	14	15	6.8	1.6	4.3
21–53s	4	13	.3	1.2	.3
21–56s	11	21	3.0	1.7	1.8
21–60s	6	22	1.0	1.6	.6
21–29×30	8	19	1.3	1.5	.9
21–30×28	10	36	1.5	2.0	.8
21–30×29	13	36	.8	2.0	.4
21–41×44	6	13	1.3	1.3	1.0
21–44×41	5	13	.5	1.2	.4
Total	157	444	6.8	7.2	.9
Calculated	150	451			

IN WILD CABBAGE

As was stated in a previous article,[13] *Fusarium conglutinans* is apparently confined in its parasitic relations to forms of *Brassica oleracea*. The wild form of this species is native to certain parts of Europe where yellows does not occur. A small sample of seed of wild cabbage was secured from England. Of 17 plants grown on infested soil, 1 succumbed to yellows. From the remaining healthy plants, 3 were saved and selfed seed were secured from each of them. These progenies were tested on the trial grounds in 1926, 1927, and 1928. It will be seen from the data given in Table 16 that each of these segregated as monohybrids. This shows that in the wild subspecies resistance is inherited in the same manner as in certain cultivated varieties of cabbage.

[13] WALKER, J. C., and WELLMAN, F. L. A SURVEY OF THE RESISTANCE OF SUBSPECIES OF BRASSICA OLERACEA TO YELLOWS (FUSARIUM CONGLUTINANS). Jour. Agr. Research 37: 233–241, illus. 1928.

TABLE 16.—*Occurrence of yellows in selfed progenies of wild cabbage plants when grown upon infested soil*

Progeny No.	Year tested	Number of plants		Deviation	Probable error	Dev. / P. E.
		Diseased	Healthy			
WC-1	1926	6	17	0.3	1.4	0.2
	1927	7	13	2.0	1.3	1.5
	1928	12	37	.3	2.0	.2
Total		25	67	2.0	2.8	.7
WC-2	1926	1	7	1.0	.8	1.3
	1927	6	12	1.5	1.2	1.3
Total		7	19	.5	1.5	.3
WC-3	1926	4	21	2.3	1.5	1.5
	1927	9	51	6.0	2.3	2.6
	1928	10	21	2.3	1.6	1.4
Total		23	93	6.0	3.2	1.9
Grand total		55	179	3.5	4.5	.8
Grand total calculated on 1:3 ratio		58	176			

DISCUSSION

The main purpose of this study was to secure more exact information about the heredity of resistance to the attack of *Fusarium conglutinans* in cultivated forms of cabbage. The evidence as a whole leads to the conclusion that in the varieties studied the majority of the plants are homozygous for susceptibility. There are practically always a few plants that resist the parasite under very favorable conditions for the development of disease. Such individuals are usually heterozygous, and when they are selfed, and the next generation tested, there is a segregation of approximately 25 per cent susceptible to 75 per cent resistant individuals. Further selection from the resistant plants yields progenies that are completely resistant under the natural field conditions employed.

Starting with homozygous susceptible and homozygous resistant individuals as parents, the F_1 hybrids show resistance equal to the resistant parent. When such hybrids are carried to the F_2 generation, segregation is that characteristic of a monohybrid, the actual counts showing segregation of approximately 3 resistant to 1 susceptible. When the F_2 generation was grown on noninfested soil to avoid elimination of susceptible individuals and plants were tested as to their genetic constitution by growing of F_3 progenies or of back crosses to susceptible plants, they were shown to consist of approximately one-fourth homozygous susceptible, one-half heterozygous, and one-fourth homozygous resistant.

In the plants studied, which included four varieties of cultivated cabbage and the wild cabbage, all evidence points to the conclusion that resistance is governed by a single dominant Mendelian factor. The extension of this study to the other cultivated subspecies of *Brassica oleracea*, such as cauliflower, kale, kohlrabi, and Brussels sprouts, would be of interest. All varieties of these so far tested show segregation into classes resistant and susceptible to the yellows organism.[14]

[14] WALKER, J. C., and WELLMAN, F. L. Op. cit

While the evidence so far secured has been consistent with an assumption of single-factor inheritance, it is not implied that this is necessarily true throughout the host range of this parasite. In fact, it does not preclude the possibility of other types of resistance than the one herein described, nor is the writer able, at the present time, to explain with complete satisfaction the variation that was consistently observed in the rate at which the disease developed in the so-called susceptible populations. In the case of a disease which is so profoundly affected by environment, one would expect to find an explanation of part of this variation in environmental rather than in genetic factors. An extensive study of a number of factors influencing the expression of resistance and susceptibility has been made,[15] and it is quite evident from the results thereof that a part of the variation referred to is due to environment and to the age of the plants when exposed to the parasite, but it is equally clear that all of the observed differences can not be so explained. In fact, as more observations are made and more data accumulate it appears increasingly likely that intermediate forms may be isolated. It is reasonable to suppose that hereditary factors acting quite independently of the main gene determining resistance may modify the expression of the disease. This is indicated by the results obtained with a comparatively few susceptible progenies recently acquired; though these show practically 100 per cent diseased plants under field conditions, a majority of them are only mildly affected, while other progenies in adjacent rows succumb promptly and completely. The first-named type might be designated as "mildly susceptible under field conditions." Its lack of true resistance is shown, however, when exposed to an environment somewhat more favorable to the parasite (constant soil temperature of 23°–24° C.), where the young plants succumb completely, while those in progenies designated as homozygous for resistance remain perfectly healthy.

The importance of more study of these finer differences is paramount from a practical standpoint. In the development of resistant varieties the lack of very close scrutiny may easily lead to the selection and perpetuation of mildly susceptible types, particularly where mass selection is practiced and where variations in soil infestation by the parasite and in seasonal climatic conditions preclude uniformly severe testing for resistance. Thus so-called resistant varieties may contain considerable percentages of such mildly susceptible individuals which remain healthy in certain seasons and in certain localities, but become diseased when exposed to an environment slightly more severe. It is strongly suspected that the mass-selected resistant varieties of cabbage, such as Wisconsin Hollander, do contain an appreciable percentage of individuals of this sort. In the light of the present findings it should be possible to improve such varieties by self-pollination and the elimination of "mildly susceptible" plants.

The possible variation in the pathogenicity of the parasite has not been overlooked. Since this is being made the subject of a special study by L. M. Blank, it is not necessary to discuss it in detail here, except to note that *Fusarium conglutinans* is quite constant in its pathogenicity so far as the study of numerous strains from widely separated localities indicates.

[15] WALKER, J. C., and SMITH, R. Op. cit.

In this study no evidence has been acquired that resistance is linked with any of the important type characters, such as color of foliage, shape of head, length of stem, vigor of plants, and time of maturity. It therefore seems reasonable to expect that no serious difficulty will be encountered in securing yellows-resistant strains that conform closely to the standard varieties already adapted to the needs of various markets.

SUMMARY

This paper reports a study of the nature of inheritance of resistance to the yellows organism (*Fusarium conglutinans*) in a number of cabbage varieties and in wild cabbage.

The symptoms of the disease and the method of testing progenies in the field for resistance and susceptibility are described.

Homozygous resistant and homozygous susceptible lines were selected first from All Head Early and Glory of Enkhuizen varieties. F_1 hybrid progenies from crosses between resistant and susceptible individuals showed resistance equal to the resistant parental lines.

In the F_2 progeny tests the segregation was, in practically all cases, a very close fit to the ratio of 3 resistant to 1 susceptible expected from a monohybrid. Back crosses between F_1 hybrid plants and susceptible plants yielded progenies which segregated close to the expected 1 to 1 ratio. A group of F_2 plants grown on noninfested soil and studied as to their genetic constitution by F_3 progeny tests, and by back crossing to susceptible plants, was shown to consist of approximately 25 per cent homozygous resistant, 50 per cent heterozygous, and 25 per cent homozygous susceptible plants.

Studies of the Jersey Wakefield variety, the Copenhagen Market variety, and wild cabbage show that in these three cases resistance to yellows is also controlled by a single gene.

The practical importance of recognizing and eliminating mildly susceptible strains from mass-selected resistant varieties is emphasized. No data have so far tended to show that resistance to yellows is linked with any important type character.

EFFECT OF THE APPLE STRAIN OF THE CROWN-GALL ORGANISM ON ROOT PRODUCTION[1]

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INTRODUCTION

The excessive development of roots frequently found on malformations of the apple and variously termed "woolly knot," "crown

FIGURE 1.—A root cutting with some of the shoots removed. Inoculations were made at the base of these detached shoots. Natural size

gall," or "hairy root" has been noted by numerous investigators. In commenting on this, Riker et al.[2] reported the isolation of organisms resembling *Bacterium tumefaciens* Sm. and Town. from "naturally occurring malformations" on apple roots. These investigators made inoculations either on the underground parts of young apple trees or on the underground parts of shoots springing from the scion. They

[1] Received for publication Jan. 14, 1930; issued April, 1930.

[2] RIKER, A. J., BANFIELD, W. M., WRIGHT, W. H., and KEITT, G. W. THE RELATION OF CERTAIN BACTERIA TO THE DEVELOPMENT OF ROOTS. Science (n. s.) 68: 357-359. 1928.

concluded that the organism they used was different from the crown-gall organism in certain respects, and that these differences were such as probably to be of specific significance. Emphasis was placed on the fact that the organism used by them was "root-stimulating," and the question was raised as to the possible utilization of this organism in commercial practice.

FIGURE 2.—Seedling cut at point designated by arrow and inoculated at basal end. Natural size

Siegler [3] had reported the presence of the apple strain of the crown-gall organism in malformations on apple roots. The assumption that the organism he used was identical with the apple strain of Smith et al.[4] was based on the fact that he obtained results similar to those obtained by these workers. Riker et al.[5] did not comment on the possibility that their organism might be identical with one used by Smith and his coworkers and by Siegler, but from their report it seems probable that the so-called apple strain is identical with the organism designated by Riker et al. as "root-stimulating."

Siegler and Piper[6] concluded that the apple strain stimulated the production of root primordia in the stem tissue of the apple. Attention was called to the fact, however, that after their initiation these root primordia could be pushed into active growth, in the apparent absence of bacteria, by planting in the soil. In this way malformations known as "woolly knots" were produced. The experiments reported here are thought to furnish evidence bearing directly on the question whether or not these organisms, per se, can be considered as "root stimulating."

EXPERIMENTS

The experiments were planned to furnish information on the effect of this organism on certain tissues of the apple. Shaw [7] showed the practicability of growing apple trees from root cuttings. This suggested to the writer the idea of using the shoots from such cuttings for inoculation purposes. The roots used were seedlings of the Rasmussen variety. The root cuttings, approximately 3 inches in length from 1-year-old growth, were placed in sand and kept under such conditions as to force sprouting. When the sprouts were from 1 to 3 inches long they were excised from the root cutting, as shown

[3] SIEGLER, E. A. STUDIES ON THE ETIOLOGY OF APPLE CROWN GALL. Jour. Agr. Research 37: 301-313, illus. 1928.

[4] SMITH, E. F., BROWN, N. A., and TOWNSEND, C. O. CROWN GALL OF PLANTS: ITS CAUSE AND REMEDY. U. S. Dept. Agr., Bur. Plant Indus. Bul. 213, 215 p., illus. 1911.

[5] RIKER, A. J., BANFIELD, W. M., WRIGHT, W. H., and KEITT, G. W. Op. cit.

[6] SIEGLER, E. A. and PIPER, R. B. AERIAL CROWN GALL OF THE APPLE. Jour. Agr. Research 39: 249-262, illus. 1929.

[7] SHAW, J. K. THE PROPAGATION OF APPLE TREES ON THEIR OWN ROOTS. Mass. Agr. Expt. Sta. Bul. 190, p. [73]-96, illus. 1919.

in Figure 1. Inoculations were made with the apple strain and with other strains of the crown-gall organism by smearing the base of these sprouts on agar cultures. They were then planted in sand in 6-inch pots. In addition to the sprouts from the root cuttings, young shoots growing from seed were used. These were cut off, approximately one-eighth inch below the junction of the root and shoot tissue (fig. 2). and the bases, including the one-eighth inch of root tissue, were inoculated and planted in the same manner as the sprouts obtained from the root cuttings. The controls received no inoculations, but it should be noted (Table 1) that certain other strains of the crown-gall organism, such as Nos. 491 and 637, also served as controls for the apple strain. Strains 491 and 637 cause the smooth type of malformation which the writer [8] has previously illustrated and which is in sharp contrast to the woolly-knot type of malformation caused by the apple strain. In previous experiments, inoculations had been made with the apple strain on 3-inch to 5-inch apple seedlings at the

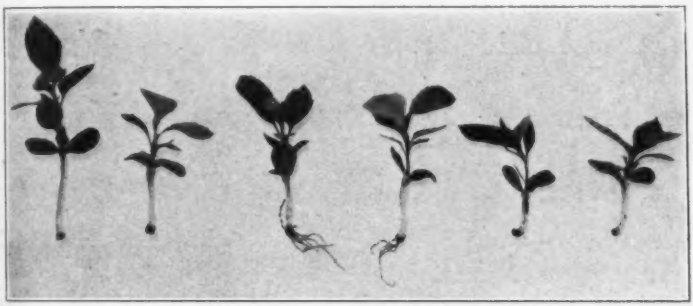

FIGURE 3.—The lot of apple seedlings in experiment 270, showing root inhibition as a result of inoculation with the apple strain. × ½

ground line, with results in agreement with those reported by Riker et al.,[9] in that an apparent root stimulation is produced when inoculations are made in this region by punctures.

DISCUSSION OF RESULTS

Reference to Table 1 shows that the apple strain of the crown-gall organism which produces the woolly-knot type of crown gall [10] with its usual production of large amounts of roots failed to stimulate root development on these shoots which might be considered as soft-wood cuttings of the apple. On the contrary, in all experiments except one (No. 296), the action was decidedly a root-inhibiting one. The two lots of shoots which were dug in experiment 270 are shown in Figures 3 and 4, which illustrate the apparent root-inhibiting effect when inoculations are made with the apple strain (fig. 3) as compared with the uninoculated control lot (fig. 4). The injurious effect of the organism on the growth of the seedlings is also shown in this comparison. That this organism did not form merely a mechanical barrier

[8] SIEGLER, E. A. THE WOOLLY-KNOT TYPE OF CROWN GALL. Jour. Agr. Research 39: 427–450, illus. 1929. (Fig. 6.)
[9] RIKER, J. A., BANFIELD, W. M., WRIGHT, W. H., and KEITT, G. W. Op. cit.
[10] SIEGLER, E. A. Op. cit. (See footnotes 3 and 8.)

TABLE 1.—*Results obtained by inoculating apple cuttings and seedlings with the apple strain and other strains of the crown-gall organism*

Experiment No.	Inoculum	Origin of shoots	Date inoculated and planted	Date dug	Number of shoots		Results			Percentage of surviving shoots showing root development
					Planted	Surviving	Number of shoots showing root development		Number of shoots showing no root development	
							Sparse	Thick		
			1929	1929						
227–A	Apple strain 486–107	Root cutting	Mar. 9	Apr. 18	15	8	0	0	8	0
227–B	491–108 control	do	do	do	15	9	2	4	3	66
228–A	Apple strain 486–107	do	do	do	15	10	0	0	10	0
228–B	Uninoculated control	do	do	do	15	9	3	5	1	89
269–A	Apple strain 486–107	do	May 10	June 10	15	11	6	2	3	73
269–B	Uninoculated control	do	do	do	15	13	0	13	0	100
270–A	Apple strain 486–107	do	do	do	10	6	2	0	4	33
270–B	Uninoculated control	do	do	do	10	7	1	5	1	86
296–A	Apple strain 486–107	Seed	May 23	July 2	10	7	0	7	0	100
296–B	Uninoculated control	do	do	do	10	7	0	7	0	100
308–A	Apple strain 486–107	do	June 18	July 11	10	6	1	1	4	33
308–B	Uninoculated control	do	do	do	10	7	3	4	0	100
308–C	491–108 control	do	do	do	10	10	0	9	1	90
310–A	Apple strain 486–107	do	June 20	do	10	7	1	0	6	14
310–B	Uninoculated control	do	do	do	10	8	1	5	2	75
310–C	Peach strain control	do	do	do	10	9	1	8	0	100
310–D	637–109 control	do	do	do	10	7	2	5	0	100
	Total apple strain 486–107				85	55	10	10	35	36
	Total uninoculated controls				70	50	8	35	7	86
	Total 491–108 controls				25	19	2	13	4	79
	Total peach strain controls				10	9	1	8	0	100
	Total 637–109 control				10	7	2	5	0	100

to root production is indicated by results obtained when other strains of the crown-gall organism were used. In these cases root development proceeded the same as on the uninoculated controls. Of the 55 shoots surviving in the lots inoculated with the apple strain, only 36 per cent showed root development, whereas of the 50 shoots surviving in the uninoculated lots, 86 per cent showed root development.

In contrast to these results, there appears to be a root stimulation following inoculations with this organism in regions other than those described. Figure 5 shows the excessive root development usually obtained as a result of inoculations on 3-inch to 5-inch seedlings at a point near the collar. Thus the nature of the results obtained depends upon the region inoculated, and it is questionable which of these two regions should be considered the criterion of the "root-stimulating" or "root-inhibiting" powers of this organism. In general, the action of organisms or other factors on cuttings of other

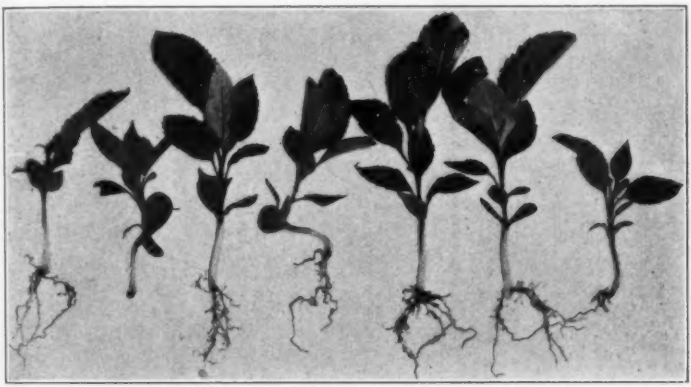

FIGURE 4.—The uninoculated control lot of apple seedlings in experiment 270, showing normal root developement. × ½

hosts would probably be the standard by which they would be judged. In these experiments the sprouts from the root cuttings correspond most closely to what are termed "soft-wood cuttings" in commercial practice. Viewed from this standpoint, the organism actually inhibits root development.

It is difficult to explain why an organism will cause apparent root stimulation when introduced into one region of a root and what apparently is an inhibiting action when introduced into another region. The two regions at which the inoculations were made, however, can hardly be considered analogous, especially from the standpoint of elaborated food and food materials. There is probably a greater concentration of elaborated food at the base of the excised shoot than there is farther up the stem. The writer has advanced the opinion that the food supply may be an important factor in connection with the occurrence of the woolly-knot type of crown gall. In fact, it was to test this hypothesis that the idea was conceived of performing the experiments described here, whereby the food supply received by the bacteria from the plant would consist of elaborated food only, without the usual accompaniment of transported food materials. There are

numerous other factors which undoubtedly influence the interaction of the organism and the host at the points of inoculation and it should be recalled that woolly knots, practically free of root developments, are frequently encountered.

The frequently observed phenomenon that girdling results in the production of roots above the girdled area prompts the explanation that the bacteria may, in some direct or indirect way, cause a girdling action when inoculated at a point some distance up from the base of the shoot. Because of its plausibility, this explanation is offered as to why root production results when inoculations are made part way

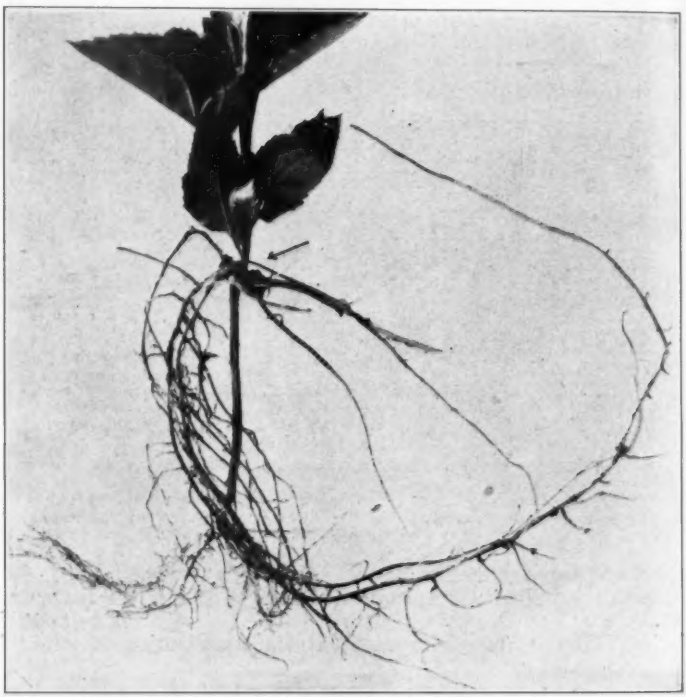

FIGURE 5.—Root production following inoculation with the apple strain at the collar of an apple seedling. Note paucity of roots in other regions. Natural size.

up the shoot. Moreover, the seedling shown in Figure 5 is held to show "root stimulation" more apparent than real, because the excessive amount of roots at the point of inoculation is counterbalanced by the resulting paucity of roots in the remaining regions. The illustration represents what might readily have happened if mechanical girdling had taken place in this region. The region below the part indicated by the arrow can not function normally. This same condition is frequently observed on nursery trees exhibiting large "woolly knots" at the graft union, and in older trees where a mass of fine roots at the collar constitutes practically the entire root system, allowing the tree to be easily uprooted. Naturally occurring woolly-knot

types of galls, when found on the basal end of the seedling part of an apple tree, almost invariably are void of root developments, whereas woolly knots at the graft union usually exhibit profuse root developments. Thus there is close agreement between the results of these experiments and the phenomena observed in nature.

SUMMARY

Inoculations with an organism considered to be identical with the apple strain of *Bacterium tumefaciens* Sm. and Town. are reported.

When inoculations were made at the cut ends of apple shoots grown from root cuttings and at the base of apple shoots grown from seed, from which most of the root systems were removed, root production was inhibited and growth was retarded.

Since the base of the shoots obtained from root cuttings and from seeds is comparable to the base of soft-wood cuttings, the organism might be considered as inhibiting root production; but since excessive root development frequently results from inoculations at other regions, it is questionable if this organism, per se, possesses either direct root-stimulating or root-inhibiting powers.

INFLUENCE OF BORDEAUX MIXTURE ON THE EFFICIENCY OF LUBRICATING-OIL EMULSIONS IN THE CONTROL OF THE SAN JOSE SCALE[1]

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INTRODUCTION

Oil sprays are sometimes combined with fungicides, particularly with those containing copper, for the simultaneous control of insects and plant diseases. The question arises whether in such combinations the oil retains its full insecticidal efficiency. Experimental data that will throw light on this question have been accumulated at the field station maintained at Vincennes, Ind., by the Bureau of Entomology in cooperation with the Purdue University Agricultural Experiment Station.

The work here reported is of interest chiefly in connection with the dormant spraying of peach for the combined control of peach leaf curl and the San Jose scale (*Aspidiotus perniciosus* Comst.), both of which must be controlled in the dormant period. For a long time prior to 1921 this control was accomplished by the use of lime-sulphur, which ordinarily gives satisfactory control of the scale and has been for years the standard remedy for leaf curl. With the widespread adoption of oil emulsions, chiefly those made according to the formula developed by Yothers (*8*)[2] and first found to be effective in the control of the San Jose scale by Ackerman (*2*), many peach growers began to use a combination of oil and Bordeaux mixture for dormant spraying; and, though there is some conflict of opinion as to the effectiveness of the Bordeaux mixture in the control of peach leaf curl when used in combination with oil, this spray is now in fairly common use. Some growers have substituted for the usual Bordeaux mixture a solution of copper sulphate, omitting the lime.

Another common use of Bordeaux mixture is in maintaining a stable mixture when hard water must be used for diluting the oil emulsion. The calcium or magnesium salts present render the soap insoluble, liberating the oil, which then rises to the surface in a continuous layer. Yothers (*8*, revised) found that this difficulty could be overcome by diluting the soap-oil emulsion with a weak Bordeaux (½–½–50 or ¼–¼–50).[3] This prevents the separation of the oil, although a distinct reaction does occur which will be discussed later.

Bordeaux mixture has also been found to be a good emulsifier (*7, p. 2011*), some growers preparing the spray in the tank of the spray rig and emulsifying the raw oil with Bordeaux mixture by the action of the pump and the agitator. Oil emulsion is also sometimes in

[1] Received for publication Dec. 4, 1929; issued April, 1930.

[2] Reference is made by number (italic) to "Literature cited," p. 765.

[3] The usual practice is followed in referring to the various formulas for Bordeaux mixture. The first term gives the quantity of copper sulphate (in pounds), the second the quantity of hydrated lime (in pounds), and the third the total quantity of spray liquid (in gallons). For brevity, copper sulphate solution alone is referred to in the tables as 1-0-50 or 2-0-50 Bordeaux. The oil concentrations mentioned in this article refer to the percentage by volume of oil present in the completed spray mixture.

emergencies added to summer sprays of Bordeaux mixture in the attempt to obtain a partial control of the scale on apple.

Most of the published statements indicate that Bordeaux mixture does not lessen the insecticidal efficiency of an oil spray with which it is combined. An exception to this general opinion is a report by Harman (*5*) showing a decreased kill of cottony peach scale when Bordeaux mixture was added to the oil spray.

The comparative tests against the San Jose scale on which the published statements are based appear to have been made with oil concentrations high enough for all the mixtures to give practically perfect control. Differences between materials may be overlooked, however, unless the tests include dilutions below the point of full effectiveness.

REACTIONS

When a soap-oil emulsion is combined with Bordeaux mixture the soap appears to react at once with the calcium or copper present, forming an insoluble calcium or copper soap. Instead of being liberated, however, the oil remains intimately associated with the Bordeaux mixture, and the two together tend to form a definite layer separate from the water in the diluted spray mixture. This separation, or "layering," is of no consequence in ordinary spraying work, since present-day outfits provide sufficient agitation to maintain a uniform mixture as long as the oil alone has not formed a separate layer.

When copper sulphate alone is added to a soap-oil emulsion, a similar reaction occurs. The resulting insoluble copper soap remains in a finely divided condition, and seems to give a sufficient volume of solid material to prevent the immediate separation of the oil. The volume of copper soap is so small, however, that the mixture layers as described above very rapidly, and vigorous agitation is needed to maintain a uniform emulsion.

EXPERIMENTAL METHODS

The experiments here reported were conducted entirely in the orchard, under field conditions, since the San Jose scale does not lend itself readily to laboratory manipulation. As the spread of the scale from tree to tree is comparatively gradual and depends wholly on chance transportation by wind or other agencies, there was no necessity for treating large areas. In the dormant season, when no movement of scales could occur, the treated areas ranged from a single, large, well-infested branch to four entire trees. In summer tests, from two to four trees were used in each plot. The materials were applied with a power outfit, or with a large hand pump which gave a pressure of approximately 150 pounds and delivered a spray very similar to that obtained with a regulation power outfit.

Except in the earliest experiments, three or four concentrations of oil were included in the test of each combination. Some of the concentrations were well below the point where complete kill was to be expected, in order that a toxicity curve could be worked out for each material. A curve of this nature gives much more complete information about an insecticide than would the result of a single test, since it gives an indication of the approximate concentration at which full effectiveness is reached.

Beginning with the tests conducted during the winter of 1926–27, the actual oil content of each dilution was determined by the method suggested by Griffin and Richardson (*4*). The samples were taken directly from the nozzle, usually one sample at the start of the spraying and a second at the close. The agreement between the two samples was almost without exception satisfactory. This procedure has been found particularly valuable in field work with oil sprays, since a number of factors conspire to cause inaccuracies in mixing small quantities of spray material in a large power outfit. The irregularities revealed by these analyses render it difficult to make direct comparisons from the control data given in Tables 3 and 4, and much better comparisons may be made from the toxicity curves in Figures 3 and 4.

The differences in efficiency caused by the addition of Bordeaux mixture first appeared in tests conducted during the growing season, but summer work was soon dropped, as the use of oil in the summer for the control of the San Jose scale is resorted to only in emergency cases on apple and practically never on peach. Experiments in the control of the scale during the growing season are extremely unsatisfactory, on account of the irregularity of spray coverage caused by the interference of the foliage and the rapid changes which occur in the relative numbers of scales of different ages during the interval between spraying and the recording of results.

In scoring the tests of summer treatments, all the scales found were counted except the very small ones which had evidently appeared after the materials were applied. In the dormant tests, only the partially grown, hibernating forms were counted; the more nearly mature scales and those which are born just before the trees become dormant never survive the winter in southern Indiana and could therefore be disregarded. Material for counting was obtained at random from various portions of the branch, tree, or block treated, where the scale population was sufficiently great to permit satisfactory scoring. The counts were usually divided about evenly between old, roughened wood, where conditions approached incrustation, and the young growth where the scale was usually less abundant. Extremely heavy incrustations were not included, since an exact count under such conditions is very difficult, although it is realized that a somewhat higher concentration of oil is needed to penetrate the scale coverings when there is a great deal of overlapping, as has been shown by Bliss[4] in the case of the camphor scale. These experiments, however, were an attempt to measure the influence of Bordeaux mixture on the effectiveness of the oil rather than to ascertain the exact dilutions needed in commercial spraying.

Summer tests were scored from 10 to 14 days after the sprays were applied. During the dormant season, however, the dead scales dry out very slowly, and it is sometimes as much as three months before they can be distinguished from the live ones with any degree of certainty.

In most of the experiments at least 1,000 scales were counted for each treatment, but in the spring of 1925 the normal mortality reached the unusually high mark of 79 per cent, so during that season the size of the counts was doubled, 2,000 scales being the standard. As the normal mortality varied considerably from year to year, that which

[4] Bliss, C. I. Unpublished manuscript.

could be attributed to the material applied has been calculated for all sprayed plots by the usual methods (*1*), and is referred to as "per cent efficiency." This permits the combining of the results from different sets of experiments on a fairly comparable basis.

OILS USED

Three different lubricating oils, the specifications for which are given in Table 1, were used in the tests reported here. All were of the general type used in preparing oil sprays. Oil No. 1 is the one in most common use for dormant spraying in the Middle West, and for that reason was used in most of the experimental work.

TABLE 1.—*Specifications* [1] *for the lubricating oils used in the experimental sprayings, Vincennes, Ind., 1923 to 1928*

Oil No.	Density at 20° C.	Volatility (4 hours at 105° C.)	Viscosity (Saybolt at 100° F.)	Unsulphonated residue	Base
		Per cent	*Seconds*	*Per cent*	
1	0.880–0.887	0.33–1.01	96–108	64.4–65.6	Paraffin or mixed.
2	.899	.010	229	56.4	Do.
3	.922	.88	310	59.2	Naphthalene.

[1] Analyses made by the Insecticide and Fungicide Board (now a part of the Food, Drug, and Insecticide Administration), U. S. Department of Agriculture.

EXPERIMENTAL DATA

SUMMER TESTS

Three experiments, all on apple, were conducted during the summer of 1923, in which oil No. 1 was used, and the materials applied with a power outfit.

The first tests were conducted on Grimes Golden during the early part of July, when the most advanced first-brood scales were about three-fourths grown. In this experiment, counts of equal numbers were made on both twigs and fruit, and the resulting figures combined. The oil was used at concentrations of 1 and 2 per cent, with two strengths of Bordeaux mixture—½–½–50 (used because of the hardness of the water) and 2–3–50. The second test was a repetition of the first, but made on Winesap trees late in July when the very earliest crawlers of the second generation were appearing. During the period when these two experiments were being conducted the temperatures were very high, reaching 95° F. or more nearly every day.

The third experiment was started in the middle of September, when the temperatures averaged much lower. At this time crawlers of late broods were appearing daily. In this test the counts were restricted to the twigs.

The data obtained in these three experiments are shown in graphic form in Figure 1.

During the summer of 1924 similar experiments were conducted on apple.[5] Two oils were used, each with ½–1–50 and 3–5–50 Bordeaux and without Bordeaux mixture. The series with oil No. 1

[5] The senior author was assisted during the summer of 1924 by B. E. Montgomery.

was repeated three times, and since these three tests were conducted within a very short period in the same orchard, and since the results of the individual tests were very similar, the three tests have been combined and reported as a single one.

The varieties in this orchard were mixed, and it was impossible to use the same variety for the entire series, but counts of scales from the different varieties revealed no significant differences in mortality. In most cases one Grimes Golden tree and one of another variety

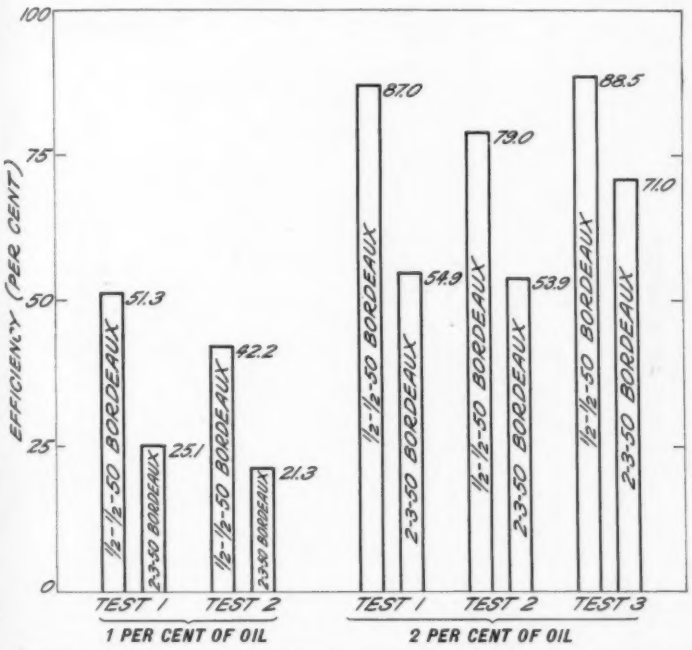

FIGURE 1.—Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the summer control of the San Jose scale on apple, Vincennes, Ind., 1923. Oil No. 1 was used throughout, emulsified with potash fish-oil soap by the boiling process. The counts include scales in all stages of development

were used for each portion of the test. The materials were applied with the hand outfit already described.

The results of these tests are summarized graphically in Figure 2.

TESTS DURING THE DORMANT SEASON

Previous to 1925 all tests made at Vincennes during the dormant period included only concentrations of oil so high that all gave practically complete control. In the spring of 1925, however, concentrations as low as 1 per cent of oil were included in the tests. Oil No. 1 was used without Bordeaux mixture, with a ½–1–50 Bordeaux, and with a 4–6–50 Bordeaux. The materials were applied to peach trees with a power outfit, and from one to four trees were included in each plot.

The data obtained are shown in Table 2, and these in part have also been combined with results of later experiments with the same oil in plotting the curves shown in Figure 4.

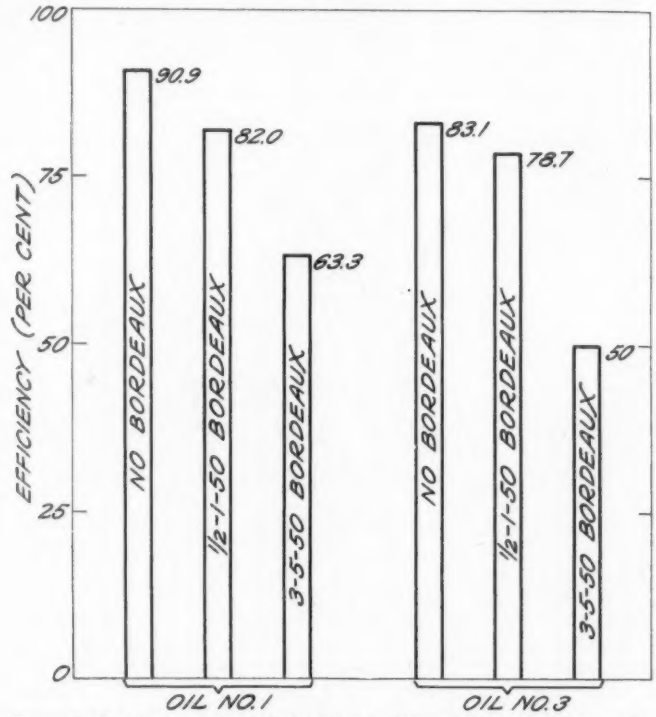

FIGURE 2.—Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the summer control of the San Jose scale on apple, Vincennes, Ind., 1924. The oils were emulsified with potash fish-oil soap by the boiling process, and used at 2 per cent in all the tests. The counts include scales in all stages of development

TABLE 2.—*Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., spring of 1925*

[Oil No. 1, emulsified with potash fish-oil soap, by the boiling process, was used throughout]

Plot No.	Strength of Bordeaux mixture	Strength of oil used	Scales counted	Scales dead	Efficiency of spray
		Per cent	*Number*	*Per cent*	*Per cent*
1	None used	1.0	2,018	99.9	99.5
2		1.5	2,014	99.4	97.1
3		2.0	2,045	100.0	100.0
4		3.0	2,062	100.0	100.0
5	½-1-50	1.0	2,026	95.9	80.3
6		1.5	2,045	97.9	89.9
7		2.0	2,030	99.9	99.5
8		3.0	2,013	99.7	98.6
9	4-6-50	1.0	2,022	93.6	69.2
10		1.5	2,048	99.1	95.7
11		2.0	2,036	99.8	99.0
12		3.0	2,064	100.0	100.0
13	Check (no treatment)		2,030	79.2	

TABLE 2.—*Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., spring of 1925*—Continued

SIGNIFICANCE OF DOUBTFUL DIFFERENCES [1]

Plots compared	Arithmetic difference	P. (probability that the difference was accidental)
1 and 5	19.2	Less than 0.01.
2 and 6	7.2	Less than 0.10.
5 and 9	11.1	Do.
6 and 10	5.8	0.10.

[1] As an aid in evaluating the data, the significance of certain critical differences has been tested by means of the statistical treatment outlined by Fisher (*5, sec. 21.1*) for determining the degree of significance to be assigned to differences between means. (Many of the differences in this paper are so great as to be obviously significant, while others are so slight as to be evidently without meaning; the statistical tests have been used chiefly with border-line cases, and have been worked out from the original counts of the random samples of which the figures given in the fourth column are the averages.) The results of these statistical tests are given in connection with Tables 2, 3, and 4. "P." indicates the probability that the difference in question could have occurred in the course of random sampling of the same lot of material. P. values greater than 0.05 ordinarily indicate that the difference in question can not be considered significant. As the value of P. drops to 0.02 or 0.01, an increasing degree of confidence may be placed in the results.

By the winter of 1926–27 a few growers were doing away with the use of soap and were emulsifying the raw oil in the spray tank with freshly made Bordeaux mixture. A few were also using the soap-oil emulsion with copper sulphate solution instead of with the usual Bordeaux mixture of copper sulphate and lime. These combinations were included in the tests of that winter, which were made on peach, the materials being applied with the hand outfit. Some of the oil concentrations were made as low as 0.6 per cent, and for the first time the proportion of oil present in the spray mixture was checked by the modified Babcock test. The data from this series of tests are given in Table 3, and have also been used in the preparation of the curves in Figure 4.

TABLE 3.—*Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., winter of 1926–27*

(Oil No. 1 used throughout)

Plot No.	Method of emulsification	Strength of Bordeaux mixture	Strength of oil	Scales counted	Scales dead	Efficiency of spray
			Per cent	*Number*	*Per cent*	*Per cent*
1	Soap, heat	None.	0.6	1,021	98.8	96.1
2			1.2	1,001	99.2	97.4
3			1.8	1,000	100.0	100.0
4	do	1-0-50	.6	1,000	98.2	94.2
5			1.2	1,000	98.5	95.1
6			1.9	1,000	100.0	100.0
7	do	½-½-50	.7	1,068	98.8	96.1
8			1.0	1,011	99.5	98.4
9			1.7	1,000	99.9	99.7
10	do	4-6-50	.8	1,000	91.4	72.2
11			1.2	1,000	95.5	85.4
12			1.8	1,000	99.1	97.1
13	Tank mix	4-6-50	.7	1,000	84.6	50.2
14			1.1	950	84.4	49.5
15			1.6	1,026	96.8	89.6
16	Check, no treatment			4,739	69.1	

TABLE 3.—*Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., winter of 1926–27*—Continued

SIGNIFICANCE OF DOUBTFUL DIFFERENCES[1]

Plots compared	Arithmetic difference	P. (probability that the difference was accidental)
2 and 11	12.0	Less than 0.01.
2 and 15	7.8	Do.
3 and 12	2.9	Approximately 0.05.

[1] See footnote to Table 2.

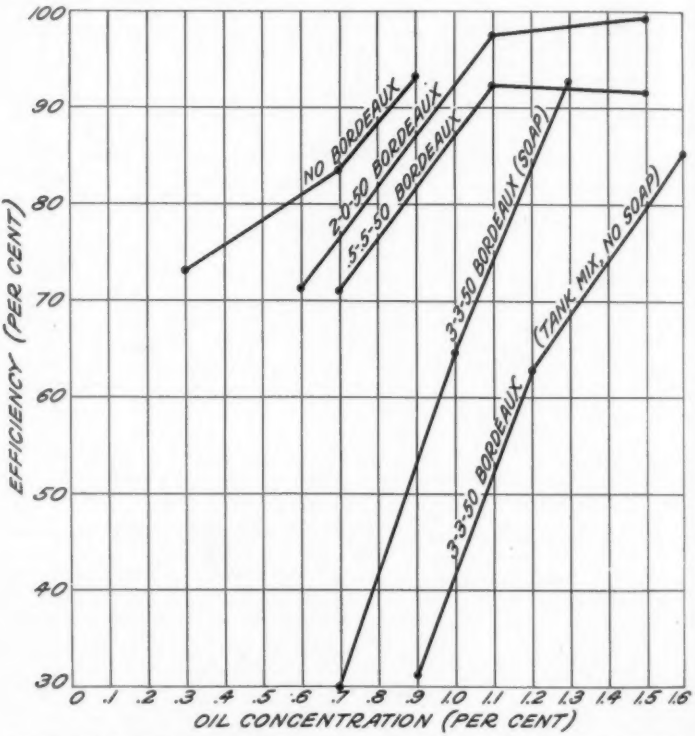

FIGURE 3.—Influence of Bordeaux mixture on the effectiveness of oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., winter of 1927–28. (See Table 4, oil No. 2)

One more repetition of this series of tests was made during the winter of 1927–28, with some concentrations of oil a little lower than those used the previous season. Applications were made to peach with a power outfit. All but one of the tests were in duplicate with the exception that a different oil was used in the second case. The data from this series are presented in tabular form in Table 4. The results of the tests in which oil No. 2 was used are plotted in Figure 3.

TABLE 4.—*Results of experiments to determine the influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., winter of 1927–28*

Plot No.	Oil used	Method of emulsification	Strength of Bordeaux mixture	Strength of oil	Scales counted	Scales dead	Efficiency of spray
				Per cent	*Number*	*Per cent*	*Per cent*
1	Oil No. 1	Soap, heat	None.	0.4	1,000	94.6	87.3
2				.9	1,500	97.7	94.6
3				1.5	1,350	96.4	91.5
4	do	do	½–½–50	.6	1,000	86.3	67.6
5				1.0	1,000	97.2	93.4
6				1.2	1,000	97.8	94.8
7	do	do	3–3–50	.5	1,000	86.4	67.9
8				.8	1,000	89.0	74.1
9				1.2	1,000	97.3	93.6
10	do	Tank mix	3–3–50	.8	1,000	89.6	75.5
11				1.0	1,000	92.9	83.3
12				1.5	1,000	96.1	90.8
13	Oil No. 2	Soap, heat	None.	.3	1,000	88.6	73.1
14				.7	1,000	93.0	83.5
15				.9	1,000	97.1	93.2
16	do	do	2–0–50	.6	1,000	87.8	71.2
17				1.1	1,000	98.9	97.4
18				1.5	1,000	99.6	99.1
19	do	do	½–½–50	.7	1,000	87.7	71.0
20				1.1	1,000	96.7	92.2
21				1.5	1,450	96.4	91.5
22	do	do	3–3–50	.7	1,000	70.3	30.0
23				1.0	1,000	85.0	64.6
24				1.3	1,000	96.9	92.7
25	do	Tank mix	3–3–50	.9	1,000	70.8	31.1
26				1.2	1,650	84.2	62.7
27				1.6	1,000	93.7	85.1
28	Check				2,000	57.6	

SIGNIFICANCE OF DOUBTFUL DIFFERENCES[1]

Plots compared	Arithmetic difference	P. (probability that the difference was accidental)
1 and 7	19.4	Less than 0.01.
2 and 11	11.3	Do.
14 and 19	12.5	Less than 0.02.
21 and 27	6.4	Approximately 0.20.

[1] See footnote to Table 2.

The date for all of the tests in which oil No. 1 was used, without Bordeaux mixture and with full-strength Bordeaux mixture (Tables 2, 3, and 4), have been combined in plotting the curves shown in Figure 4. Since in all cases the oil reached practically its full toxicity at a concentration of 2 per cent, the graph shows the results only as far as this dilution. The usual method of least squares has been used in plotting the curves.

Preliminary experiments to determine the influence of Bordeaux mixture on one of the well-known miscible oils seemed to show a trend similar to that obtained with lubricating-oil emulsion, but these tests were not sufficiently extensive or conclusive to warrant presenting them in detail.

DISCUSSION OF RESULTS

It is evident from the data here presented that the effectiveness of lubricating oil-soap emulsion at low concentrations is reduced by the addition of ordinary strengths of Bordeaux mixture. In all

dormant tests in which the oil concentration was 1.3 per cent or less, a significant difference has appeared. The curves plotted in Figure 4 indicate that this effect may not entirely disappear until the oil concentration reaches 1.7 or 1.8 per cent, but the data available are not sufficient to establish the exact point where the differences lose their significance.

When emulsified in the tank by Bordeaux mixture, instead of by the usual soap process, the oil appeared in two series of dormant tests to suffer a further reduction in efficiency, whereas in a third series its efficiency appeared to be unaffected. The evidence therefore suggests that this process of emulsification renders the oil less effective, although the extent of this reduction can be determined only by further tests.

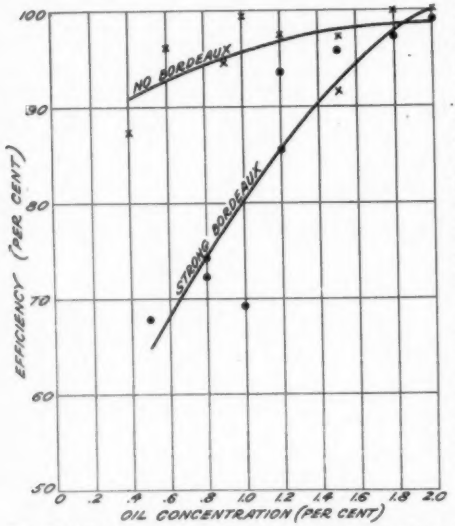

FIGURE 4.—Influence of Bordeaux mixture on oil emulsion in the dormant control of the San Jose scale on peach, Vincennes, Ind., 1925–1928. The curves were made from data brought together from all dormant spray tests in which oil No. 1 was used. (See Tables 2, 3, and 4)

When a weak Bordeaux mixture was used with the oil spray, as for the purpose of maintaining a stable emulsion with hard waters, slightly significant reductions in effectiveness occurred in some tests but failed to appear in others. The addition of copper sulphate solution appears to have a similar slightly detrimental influence, although more extensive tests are needed before definite conclusions can be drawn.

In the tests conducted during the summer the influence of Bordeaux mixture on the effectiveness of the oil was more marked than in the dormant sprays, and the differences were evident with concentrations of oil up to 2 per cent. The consistent replication of results through seven repetitions of substantially the same tests during two seasons gives the data undoubted significance.

POSSIBLE CAUSE OF REDUCTION IN EFFECTIVENESS

Although the writers have not determined experimentally in just what way Bordeaux mixture acts on the oil in the diluted spray, the following quotations from DeOng, Knight, and Chamberlin (*6, p. 362–363*) offer a plausible explanation of the loss of efficiency:

> Some emulsifiers, such as lime or kaolin and other earths, are capable of absorbing considerable amounts of oil, as well as emulsifying them. This is particularly pronounced and important when the emulsifier or spreader is used in large quantities. All such absorbed oil is unavailable for liberation as a free

liquid and constitutes, therefore, a permanent loss—assuming, of course, that free oil is the effective agent. * * *

Lime, by its absorptive capacity, tends to prevent the oil from coming into direct contact with the object sprayed, and hence serves as an inhibiting factor.

The writers believe that the Bordeaux mixture acts in a mechanical way, absorbing a certain volume of oil, thus preventing its penetration into the insect.

PRACTICAL APPLICATION

In actual practice oil is ordinarily used at a higher concentration than is necessary for perfect control with heavy and thorough application. The excess oil offsets in some measure the lightness of many of the commercial applications, although it can not make up for the missing of entire portions of trees. If the oil is used at a concentration not sufficiently above the exact point of full efficiency it is obvious that the addition of Bordeaux mixture may reduce its effectiveness to a point where incomplete control will result. In terms of orchard practice, the addition of full-strength Bordeaux mixture would warrant an increase in the oil content of the spray by 0.5 to 1 per cent of the total volume of diluted material. For example, if in dormant spraying without Bordeaux mixture, or with a very dilute Bordeaux mixture, 2 per cent of actual oil has been found to be barely enough to control the scale, the addition of a heavy Bordeaux mixture should be accompanied by an increase in the oil content to 2.5 or 3 per cent.

With trees in foliage, the oil content of the spray mixture can not always be increased with safety. In case an emergency necessitates spraying with oil for scale control in the summer, the oil should be applied separately rather than in combination with one of the regular applications of Bordeaux mixture.

SUMMARY

As oil sprays for the control of insects are sometimes combined with fungicides, an investigation has been made of the comparative efficiency of the oil emulsion in such combinations.

Lubricating-oil emulsion was found to be reduced in efficiency in the control of the San Jose scale by the addition of Bordeaux mixture at usual strengths (2–3–50 to 4–6–50). This reduction appears in dormant spraying at low oil concentrations, and practically disappears, under the conditions of these experiments, when the oil content reaches 1.5 per cent. In summer spraying, this reduction is very marked with oil concentrations up to 2 per cent.

In practical dormant spraying this renders desirable a moderate increase in the oil content of lubricating-oil emulsion whenever full-strength Bordeaux mixture is used with it. Oil applications in the summer for emergency control should be made separately rather than in combination with one of the regular Bordeaux sprays.

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STUDIES OF VITAMIN C IN FRESH AND CANNED TOMATOES[1]

By BERTHA CLOW and ABBY L. MARLATT, *Department of Home Economics, University of Wisconsin*[2]

INTRODUCTION

The value of vitamin C as an antiscorbutic has been so well established that it has become almost axiomatic to recommend raw fruit juice high in this vitamin for feeding infants and young children. Orange juice is still looked upon as the standard, but tomato juice is now being widely substituted. For this reason it becomes important to know to what extent, if at all, the vitamin-C content of canned tomatoes is affected by the different methods used in canning.

The experiments reported in this paper were carried out to answer the following questions: Are canned tomatoes as reliable as fresh tomatoes as a source of vitamin C? How long after canning are home-canned tomatoes a satisfactory source of this vitamin? If tomatoes are picked green and allowed to ripen at room temperature, will they still be rich in vitamin C? Is the vitamin-C content of greenhouse-ripened tomatoes as high as that of field-ripened tomatoes? Is the commercial method of coloring tomatoes in an atmosphere of ethylene destructive to vitamin C? Does the usual household method of making green-tomato pickles destroy vitamin C? The answers to these questions are found in this report of experimental studies carried on in the home economics research laboratory of the University of Wisconsin from 1925 to 1928.

EXPERIMENTAL PROCEDURE

The recovery method of study was used throughout the investigation. This method was preferred to the protection method, which is most frequently reported for vitamin-C work, because tomatoes of all the types studied were not available for a full 60-day feeding period. The recovery method used was as follows: Guinea pigs were fed a scurvy-producing ration plus cabbage ad libitum until they reached approximately 250 grams in weight. Then the cabbage was taken away and the scurvy ration alone was fed until positive scurvy symptoms were noted; that is, either a "jerky run" or swollen wrists. The tomato juice was then fed, and the feeding was continued for 16 days. On the seventeenth day the guinea pig was killed and an autopsy performed.

On autopsy the following conditions were looked for: Hemorrhage and swelling of the wrists or elbows of the forelegs and also below the hip joint of the hind legs, hemorrhage or "beading" at the costochondral juncture of the ribs, loose teeth, and any possible hemorrhages on the abdominal organs or on the lining of the abdominal cavity.

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[2] The authors wish to express their appreciation to Helen T. Parsons and Ella Woods for their guidance in this problem and to Ina Stevenson for her assistance in the care and feeding of the animals used.

The scurvy-producing ration is a modification of that used by Cohen and Mendel[3] and also by Parsons and Reynolds.[4] The ration consists of the following: Soybean flour, 1,620 gm.; dried yeast, 120 gm.; purified casein, 105 gm.; calcium lactate, 60 gm.; sodium chlorate, 60 gm.; filter paper, 40 gm.; and butterfat, 100 gm. The soybean flour is a commercial product, the preparation of which involves heating. The yeast also is a commercial product and is a reliable source of the vitamin-B complex. The casein was purified by soaking for a week in water slightly acidified with glacial acetic acid (about 5 c. c. per 6 quarts of tap water). This water was changed every day. The filter paper was cut into small pieces and beaten in distilled water until a fine pulp was obtained. This was poured upon the mixed dry ingredients and the mass rubbed until evenly mixed. When dry it was ground and thoroughly mixed with the melted butterfat.

The tomatoes used in these studies were obtained for the most part from the university greenhouse and gardens, though some were received from a local grocery and some from a local commercial greenhouse. They were all either Bonnie Best or a similar variety.

Two methods of canning were employed, (1) the cold-pack and (2) the open-kettle method. By the first method the tomatoes are blanched in boiling water for 3 minutes, the skins removed, and the fruits packed in sterile ½-pint jars. The jars are then put in a boiling-water bath for 20 minutes and the seal completed. By the second method the tomatoes are blanched, the skins removed, and the fruit cooked in an open keetle for 20 minutes and then put into ½-pint sterile jars and sealed.

The green tomato pickles were prepared according to a common household method as reported in popular cookbooks, except that spices and additional flavors such as onion and pepper were omitted. Sliced tomatoes (8,000 grams) and salt (400 grams) were placed in alternate layers in crockery bowls and allowed to stand 24 hours. At the end of that time the salt liquid was drained off (2,445 grams). To the tomatoes, vinegar and sugar were added and the mixture cooked in the open kettle for a total period of 55 minutes and then packed in ½-pint sterile jars and sealed.

The ethylene-colored tomatoes were grown in the greenhouse and picked when they were green but full grown. They were colored in a small air-tight chamber (an ice box) to which the proportion of ethylene to cubic-foot volume of the chamber was 1 part to 1,000. From four to eight days were required to color the tomatoes. This is perhaps a little longer than is necessary commercially, but the tomatoes showed no sign of coloring when they were put into the chamber, and they were left long enough to develop a very good red color.

EXPLANATION OF TEXT FIGURES

The initial growth period between the time that cabbage was removed from the diet and scurvy symptoms were evident is not included in the curves. The short crossline indicates the point at which a definite case of scurvy was diagnosed and the 16-day tomato feeding

[3] COHEN, B., and MENDEL, L. B. EXPERIMENTAL SCURVY OF THE GUINEA PIG IN RELATION TO THE DIET. Jour. Biol. Chem. 35: 425–453. 1918.

[4] PARSONS, H. T., and REYNOLDS, M. S. THE DEPLETION OF VITAMIN C IN THE LIVER OF THE GUINEA PIG ON A SCORBUTIC RATION. Jour. Biol Chem. 59: 731–736, illus. 1924.

period begun. The following system of letters indicates the autopsy findings at the end of the recovery period:

A, scurvy cured.
A−, scurvy practically cured.
B+, scurvy considerably cured.
B, scurvy moderately cured.
B−, scurvy very slightly cured.
C, scurvy symptoms very marked.

It should also be added that the weight curves of all animals on the same dosage of tomato although available are not included in cases where there are several animals. It was chosen rather to represent what would be considered average or typical results. The number of guinea pigs on each dosage of each kind of tomato is included in each graph. This number totals 107 exclusive of the controls. With practically each kind of tomato, however, both higher and lower dosages than those presented were actually fed. It seemed necessary to include data only on the "borderline" doses, i. e., the minimum dosage to bring about approximately complete recovery in 16 days.

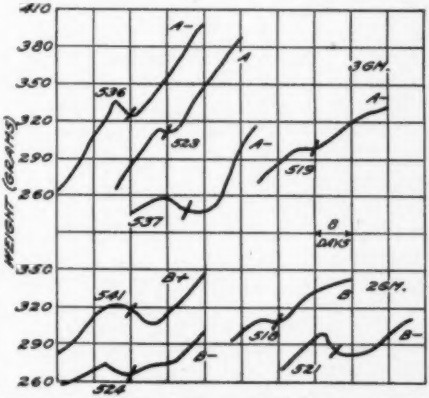

FIGURE 1.—Growth and recovery of guinea pigs on 2 and 3 gram daily doses of field-ripened raw tomatoes fed as an antiscorbutic. The system of lettering on this and the other figures is explained in the text

DISCUSSION OF RESULTS

The question of the relative importance of the character of the weight curve and the degree of recovery as shown by the autopsy must be considered. As a general rule resumption of growth accompanies recovery from the scorbutic condition. However, when one is determining the borderline dosage on which recovery is obtained there is very often resumption of growth but only partial recovery shown when autopsy is performed at the end of 16 days. These are doubtless cases where a feeding period of more than 16 days would have brought about complete recovery, but the 16-day recovery period being taken as a standard for comparison, the authors believe that the degree of recovery as shown by the autopsy is a more important criterion than the character of the weight curve.

FIELD-RIPENED RAW TOMATOES

The curves and degree of recovery as shown in Figure 1 indicate that a 2-gram daily dosage of raw tomatoes ripened in the field does not bring about recovery, but that a 3-gram dosage does bring about practically complete recovery. Six animals were fed the former and eight the latter dosage. Throughout these studies the field-ripened raw tomatoes are used as the standard of comparison, and 3 grams daily is considered the minimum recovery dosage. All comparisons are made on this basis.

FIELD-RIPENED CANNED TOMATOES FED WITHIN NINE MONTHS AFTER CANNING

The results shown in Figure 2 for 4 of the 10 guinea pigs used very clearly indicate that a 3-gram dosage of cold-pack and water-bath processed tomatoes fed within nine months after canning brings about practically complete recovery. Thus it seems that a storage period of nine months does not lessen the vitamin-C content of tomatoes canned by the cold-pack method.

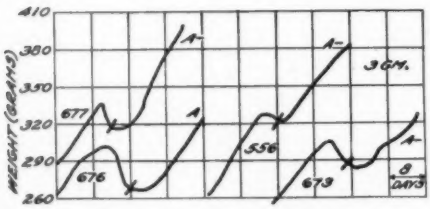

FIGURE 2.—Growth and recovery of guinea pigs on a 3-gram daily dosage of field-ripened tomatoes canned by the cold-pack, water-bath process and fed within nine months as an antiscorbutic. The tomatoes were from the same lot as those mentioned in Figure 1

The effect of the usual household method of canning tomatoes by cooking in an open kettle is shown by the results in Figure 3, which indicate that a 3-gram dosage of tomatoes canned by this method can not compare in vitamin-C content with a like dosage of tomatoes canned by the cold-pack method. In fact, even 4 grams did not always give complete recovery, although this dosage can probably be considered the minimum recovery dosage when this method is used. This loss of vitamin C is quite understandable in view of the well-known instability of vitamin C to oxidation.

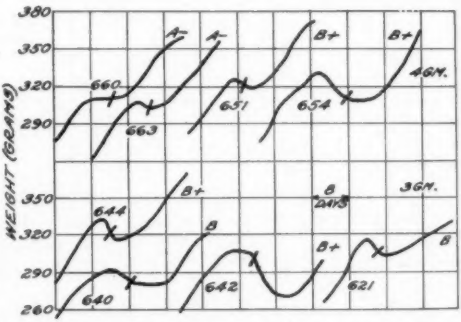

FIGURE 3.—Growth and recovery of guinea pigs on 3 and 4 gram doses of daily field-ripened tomatoes canned by the open-kettle process and fed within nine months as an antiscorbutic. The tomatoes were from the same lot as those mentioned in Figure 1

FIELD-RIPENED CANNED TOMATOES FED 15 TO 20 MONTHS AFTER CANNING

Field-ripened tomatoes were canned by the cold-pack method in September, 1926. Later that fall and also in the spring of 1927 tomatoes from this lot were fed, and 3 grams was found to be the recovery dosage. (Fig. 2.) When tomatoes from the same lot were fed in the late fall of 1927 and in the spring of 1928, that is 15 to 20 months after canning, 3 grams would not bring about recovery but a 4-gram daily dosage was necessary. (Fig. 4.) Nine animals were fed the former and six the latter dosage. Why this deterioration in storage did not appear in 8 or 9 months but was evident in 15 months can not be explained, except possibly by the fact that the summer months intervened. The canned tomatoes were not kept in a cool room but in the laboratory, where the summer temperature was often higher than 70° F. There were no signs of spoilage, however, when the jars were opened.

This loss of vitamin C during long storage of the canned product is further shown by the field-matured but green canned (cold-pack)

tomatoes. Within the first nine months after canning 5 grams was the recovery dosage. (Fig. 5, B.) Two animals were fed raw, green, field-matured tomatoes, and six raw greenhouse (full-grown) tomatoes with the same result; five were fed the full-grown green canned tomatoes. But 15 to 20 months later (the summer months having intervened) the results in Figure 6 indicate that 5 grams would not bring about the same degree of recovery.

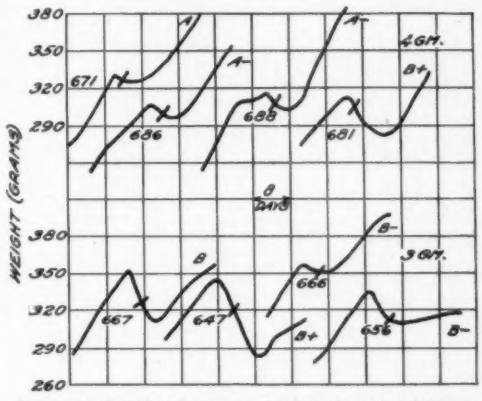

FIGURE 4.—Growth and recovery of guinea pigs on 3 and 4 gram daily doses of field-ripened tomatoes canned by the cold-pack, water-bath process, and fed 15 to 20 months later as an antiscorbutic

FIELD VERSUS GREENHOUSE TOMATOES

Greenhouse tomatoes left on the vines until thoroughly ripe were picked in one lot and kept in an electric refrigerator during the feeding period from July 13 to August 2, 1928. The records in Figure 7 show that a 3-gram dosage gave moderate recovery from scurvy and a 4-gram dosage gave approximately complete recovery. Nine animals were fed the former and four the latter dosage. By comparing these results with the standard of 3 grams for field-ripened tomatoes, it appears that greenhouse-ripened tomatoes are not quite so potent a source of vitamin C as field-ripened tomatoes. The field tomatoes were also picked in one lot and kept in the electric refrigerator during the feeding period (August 29 to September 23, 1927), so that the question of different handling of the tomatoes does not enter in as an explanatory factor.

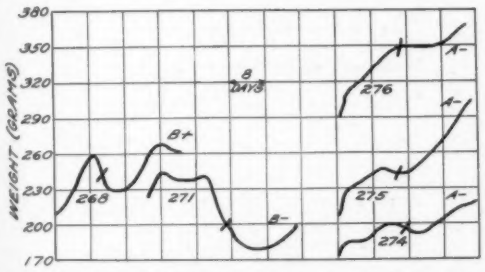

FIGURE 5.—Growth and recovery of guinea pigs on a 5-gram daily dosage of field-matured green tomatoes raw (left) and also canned by the cold-pack, water-bath process, and fed within nine months as an antiscorbutic (right)

EFFECT OF ETHYLENE COLORING [8]

A recent development in the commercial world is the use of ethylene for ripening or coloring certain fruits and vegetables. Work done at the Minnesota experiment station under the direction of R. B. Harvey has shown that practically all fruits and vegetables from the Tropical and Temperate Zones can be successfully colored with this gas. Thus the possibilities of its widespread application in the commercial world are evident. The tomatoes used in these studies were not obtained in the open market but were colored in the laboratory as described previously. After they were colored they were placed in the electric refrigerator at about 42° F. The results as shown in Figure 8 indicate that 4 grams is the recovery dosage, seven animals being fed the 3 grams and four the 4 grams dosage. Thus coloring in an atmosphere of ethylene does not entirely prevent the development of vitamin C, and moreover the vitamin seems to develop to the same extent as though the tomatoes were allowed to ripen on the vine in the greenhouse.

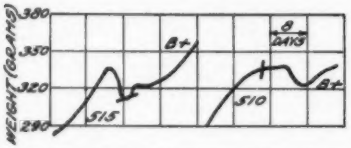

FIGURE 6.—Growth and recovery of two guinea pigs on a 5-gram daily dosage of field-matured green tomatoes canned by the cold-pack, water-bath process, and fed 15 to 20 months later as an antiscorbutic. The tomatoes were from the same lot as those mentioned in Figure 5

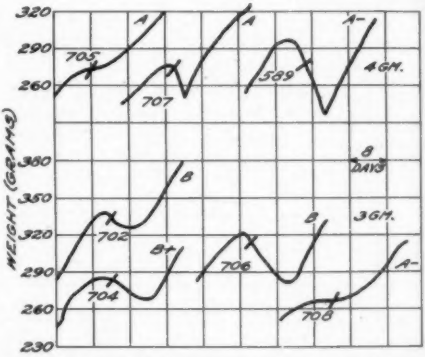

FIGURE 7.—Growth and recovery of guinea pigs on the 3 and 4 gram daily doses of greenhouse tomatoes which had been allowed to become thoroughly ripe on the vine and were then fed as an antiscorbutic

GREENHOUSE TOMATOES ALLOWED TO RIPEN IN A DARK ROOM

Full-grown but green tomatoes grown in the greenhouse were picked on April 17, 1927, and put into a dark closet at room temperature. On May 18, 1927, the tomato-feeding period was started, but it was June 9 before all the guinea pigs had received the last dose. In the meantime the tomatoes had become very soft, too soft in fact for table use. It was found in an

[8] Since this paper was put into final form for publication, the following article regarding the effect of ethylene ripening of tomatoes on the vitamin A, B, and C content has appeared: HOUSE, M. C., NELSON, P. M., and HABER E. S. THE VITAMIN A, B, AND C CONTENT OF ARTIFICIALLY VERSUS NATURALLY RIPENED TOMATOES. Jour. Biol. Chem. 89: 495–504, illus. 1929. House, Nelson, and Haber showed that tomatoes which had been picked when green and then air-ripened had the same vitamin-C content as similar tomatoes ethylene-ripened, but that both the air-ripened and ethylene-ripened tomatoes were less potent in vitamin C than the vine-ripened (greenhouse) tomatoes. Thus, they show that vitamin C does not develop in the tomato to the same extent after it is picked from the vine that it does when it is allowed to ripen on the vine. House, Nelson, and Haber used only the 4-gram level in feeding, which is high, as shown in this paper, where the minimum recovery dosage is reported in each case. By the latter method the results seem to show that vitamin C develops practically to the same extent whether the ripening takes place on the vine, in a dark room, in a light room, or in an atmosphere of ethylene. The present studies confirm those of House, Nelson, and Haber in showing that there is an increase of vitamin C in the ripening of tomatoes and also that ethylene ripening may not be destructive to vitamin C in tomatoes, though it may to a slight extent inhibit its development.

experiment in which five animals were used that 3 grams of these tomatoes gave approximately complete recovery (fig. 9), thus showing that vitamin C develops after the tomato has been severed from the vine and allowed to ripen in a dark room.

VITAMIN-C CONTENT OF TOMATOES AT DIFFERENT STAGES OF MATURITY

As a corollary to the other studies, data were obtained on the vitamin-C content of tomatoes at different stages of maturity. Although weight curves are not included, an attempt was made to determine a recovery dosage when small, immature, green tomatoes about 1 inch in diameter from both field and greenhouse were used. In preparing these tomatoes for feeding it was necessary first to rub them over a grater or put them through a grinder and then obtain the juice by putting the pulpy material into a cheesecloth bag and squeezing it. Because of the pungent taste, it was very difficult to get the animals to take this juice.

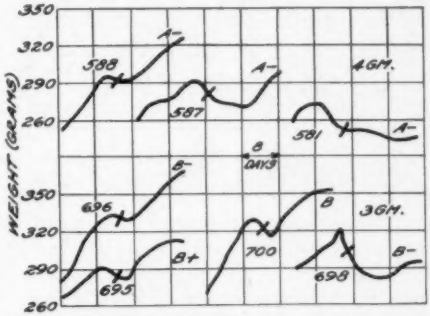

FIGURE 8.—Growth and recovery of guinea pigs on 3 and 4 grams daily doses of greenhouse tomatoes picked when mature, but green, and then allowed to develop a good, red color in an atmosphere of ethylene gas before being fed as an antiscorbutic. The tomatoes were the same lot as those mentioned in Figure 7

Five and seven gram doses failed to bring about recovery, and even the animal on the 10-gram dosage died after 9 days' feeding of raw, green, immature tomato. However, when immature field tomatoes were canned by the usual cold-pack method and fed to three guinea pigs in 10-gram doses all recovered in the 16-day period.

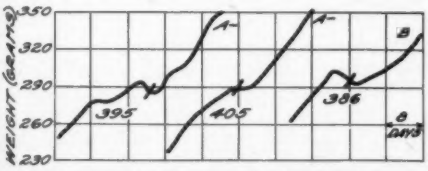

FIGURE 9.—Growth and recovery of guinea pigs on a 3-gram daily dosage of greenhouse tomotoes, picked when mature but green, and allowed to ripen in a dark closet at 70° F. before being fed as an antiscorbutic

In feeding the mature but green canned tomatoes the minimum recovery dosage is 5 grams. (Fig. 5, right.) As data have been presented showing that 3 grams of the mature ripe raw or cold-pack canned (field) tomato is the recovery dosage this seems to show that vitamin C in tomatoes develops with the maturing process.

EFFECT OF SOFTENING ON THE VITAMIN[6] C CONTENT OF TOMATOES

In a preliminary study of the effect of softening on the vitamin-C content of tomatoes, both fresh and canned green fruits were fed. It

[6] The writers show that canned green tomato is more effective in feeding (for vitamin C) than the raw, and they wish to call attention to the following publication on the vitamin-A content of asparagus: CRIST, J. W., and DYE, M. THE ASSOCIATION OF VITAMIN A WITH GREENNESS IN PLANT TISSUE. II. THE VITAMIN A CONTENT OF ASPARAGUS. Jour. Biol. Chem. 81: 525–532, illus. 1929. In the words of Crist and Dye, "* * * it is relevant to infer that the poor quality of bleached asparagus as food for the animal may not be due alone to vitamin A deficiency but also to an over-abundance of deleterious chemical compounds. This conclusion has some additional support in the fact that cooking the white asparagus improved its nutritive value to some extent; a result which could have been due to the effect of the cooking process on the chemical constituents of the tissue" (p. 531).

was found that 5 grams of canned field-matured green tomato was the recovery dosage, but that 5 grams of the raw field-matured green tomatoes would not bring about the same degree of recovery. (Compare left and right fig. 5.) Thus the canned green tomatoes seem better than the raw.

To determine the effect of softening on the vitamin-C content, raw field-matured green tomatoes were wrapped in paper and stored in a large refrigerator in one of the university buildings and allowed to remain there for three weeks. At the end of that time it was found that they had softened, and when fed to six guinea pigs at a 5-gram level they gave approximately complete recovery. (Fig. 10.) As mentioned above, 5 grams of raw field-matured green tomatoes fresh from the vine would not bring about recovery, and 5 grams of this same kind of green tomato canned by the cold-pack method did bring about recovery. As these green, raw, but stored tomatoes gave the same recovery as green canned tomatoes the difference in vitamin-C value after softening but not coloring is of interest.

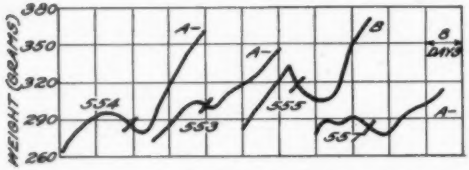

FIGURE 10.—Growth and recovery of guinea pigs fed a 5-gram daily dosage of field-matured green tomatoes, stored for three weeks at 42° F., and then fed as an antiscorbutic

Some of these stored tomatoes which had begun to turn pink were allowed to color further at room temperature (70° F.). Although they never developed a red color like that of the field-ripened tomatoes or the tomatoes allowed to ripen at room temperature without previous storage, it required only slightly more than a 3-gram dosage to bring about recovery. Four animals were fed the 3 and three the 4 gram dose. (Fig. 11.)

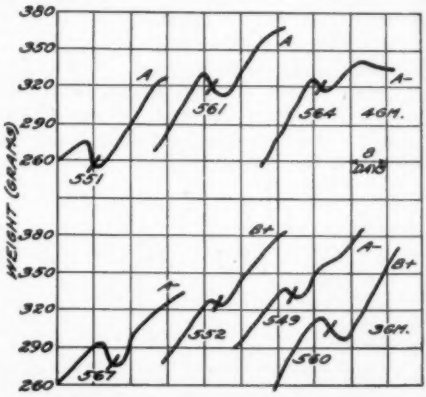

FIGURE 11.—Growth and recovery of guinea pigs on 3 and 4 gram daily doses of field-matured green tomatoes, stored for three weeks at 42° F. and then allowed to turn pink at 70° F. before being fed as an antiscorbutic. The tomatoes were the same lot as those mentioned in Figure 10

As previously stated, when greenhouse tomatoes were picked green and allowed to ripen in a dark room only a 3-gram dosage was required to bring about recovery, whereas when greenhouse tomatoes were picked from the vines when ripe a 4-gram dosage was required. The tomatoes which had been allowed to ripen after being picked were very soft when fed. Possibly in the case of the stored tomatoes the factor of loss of moisture with greater concentration of vitamin C per unit of weight should not be overlooked. However, the fact that canned green tomatoes are more effective than raw green tomatoes can hardly be explained on the basis of loss of moisture.

EFFECT OF PICKLING ON VITAMIN C IN GREEN TOMATOES

Field-matured green tomatoes were made into pickles as described previously. Some tomatoes from the same lot were canned by the usual cold-pack method and used as vitamin C controls in this study on the effect of the pickling process. Figure 5, B, shows that there is a considerable amount of vitamin C in these canned green tomatoes, as 5 grams is the minimum recovery dosage. Cooked and canned green tomato pickles were fed at levels up to and including 10 grams, but, as Figure 12 indicates, at no time was a recovery level reached. It is therefore apparent that the vitamin-C content of green tomato pickles is negligible.

SUMMARY

The recovery type of experiment was used to study the vitamin-C content of tomatoes under varying conditions as listed below. The recovery method consisted in depleting the storage of vitamin C in the guinea pigs so that definite scurvy symptoms would develop and then determining the minimum dosage of tomato that would bring about approximately complete recovery in 16 days.

The recovery dosage of field-ripened raw tomatoes was found to be 3 grams, and this was taken as the standard of comparison.

In these experiments no difference was detected in the potency of field-ripened tomatoes fed raw and those canned by the cold-pack method and fed within nine months; therefore they are believed to be essentially the same in vitamin-C content.

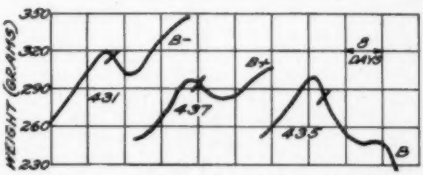

FIGURE 12.—Growth and recovery of three guinea pigs on a 10-gram daily dosage of pickles made from field green tomatoes fed as an antiscorbutic

When field-ripened tomatoes canned by the cold-pack method are used 15 to 20 months after canning, there seems to be a slight loss in the original vitamin-C content.

Field-ripened tomatoes canned by the open-kettle method and fed within nine months are not so potent as those canned by the cold-pack method.

Field-matured green tomatoes stored at a temperature of 42° F. for three weeks and then allowed to turn pink at room temperature (70° F.), and also greenhouse-matured green tomatoes allowed to ripen at room temperature without previous storage, seem to be as potent a source of vitamin C as tomatoes ripened on the vines in the field. Both types of tomato were quite soft when fed.

Greenhouse tomatoes allowed to ripen on the vine are not quite so potent a source of vitamin C as field tomatoes ripened on the vine.

Coloring of greenhouse tomatoes in an atmosphere of ethylene does not greatly alter or affect the development of vitamin C.

Vitamin C increases with maturity of the tomato. Ripening or coloring after the tomatoes have been severed from the vine does not seem to alter the development of vitamin C.

Mature green tomatoes canned by the cold-pack method are a more potent source of vitamin C than raw mature green tomatoes.

The vitamin C in green tomato pickles is negligible.

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